Biochar addition to forest plantation soil enhances phosphorus availability and soil bacterial community diversity

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Chuifan Zhou[a], Kate Heal[b], Mulualem Tigabu[c], Lidan Xia[a], Huaying Hu[a], Danyang Yin[a], Xiangqing Ma[a]*

[a]Forestry College, Fujian Agriculture and Forestry University, University Engineering Research Center of Sustainable Plantation Management, Fuzhou, 350002 Fujian Province, People's Republic of China

[b]School of GeoSciences, The University of Edinburgh, Crew Building, Alexander Crum Brown Road, Edinburgh EH9 3FF, UK

[c]Swedish University of Agricultural Sciences, Faculty of Forest Science, Southern Swedish Forest Research Centre, PO Box 49, SE-230 53 Alnarp, Sweden

*Corresponding author

Prof. Ma Xiangqing

Forestry College,

Fujian Agriculture and Forestry University,

350002 Fuzhou, Fujian Province, PR China

E-mail: lxymxq@126.com

Tel: 0086 591 83789865 (Office)

Fax: 0086 591 83780261

Abstract

Depletion of soil nutrients is a major cause of decline in productivity of forest plantations in successive rotations. Biochar amendment in agricultural systems has been shown to yield various beneficial effects, including increasing soil phosphorus (P) availability. However, the direct and indirect effects of biochar addition on forest soil P dynamics have largely been unexplored. The objective of this study was to examine how biochar produced from harvest residue (leaves and woodchips) affect the P dynamics in second rotation *Cunninghamia lanceolata* (Chinese fir) plantation soil. An incubation experiment which involved mixing of forest soil with 1% or 3% *w/w* leaf or woodchip biochar, pyrolyzed at 300 °C or 600 °C, was conducted for 80 days at 20 °C. After 7, 40 and 80 days of incubation, soil samples were analyzed for total and available P, inorganic and organic P pools, and soil phosphatase activity. At the end of the incubation period, bacterial community composition and diversity were analyzed by 16S rDNA sequencing. The leaf biochar produced at both pyrolysis temperatures was more alkaline and had significantly higher soluble P, nitrogen and calcium contents than the woodchip biochar. Soil total and available P increased significantly in all leaf biochar treatments after 80 days incubation compared to the untreated control soil, but the woodchip biochar treatments had no significant effects. At the end of the experiment, Al-P content was significantly lower and Ca_{10}-P content higher in soil amended with both biochar types compared to the control soil, and Fe-P content was significantly higher in the leaf biochar treatments. Contrary to expectations, acid and alkaline phosphatase enzyme activities were significantly lower in some of the biochar treatments after 80 days incubation compared to the control soil. Nevertheless, the diversity of the bacterial community was higher in leaf biochar-amended forest soil than the woodchip biochar-amended and untreated soil at the end of the experiment. In particular, the abundance increased in the leaf biochar soil treatments of P-solubilizing bacteria, such as *Burkholderia-Paraburkholderia*, *Planctomyces*, *Sphingomonas* and *Singulisphaera*, which can indirectly improve P availability in soil. Thus, conversion of tree harvest residues, particularly leaves, into biochar and recycling back into the soil could be a viable option to boost P availability and help

to conserve nutrients or reduce nutrient losses for the next rotation. Before recommending plantation management with biochar, long-term studies are required assessing the life cycle of biochar under field conditions and its promoting effect on growth of *C. lanceolata*.

Keywords: biochar, *Cunninghamia lanceolata*, microbial diversity, phosphate-solubilizing bacteria, phosphorus availability

1. Introduction

A decline in productivity of forest plantations under successive rotations (Tian et al., 2011) has been a major concern among forest managers. Under continuous planting on the same site, depletion of soil nutrients, particularly available phosphorus (P), which is the major growth limiting factor in the tropical and sub-tropical regions, is often associated with productivity decline (Yang et al., 2000). For instance, soil available P has been shown to decrease by more than 50% in second rotation plantation sites compared with that of first generation *Cunninghamia lanceolata* (Lamb.) Hook (Chinese fir) plantations (Wang et al., 2006). The current management practice to conserve site resources during the inter-rotation phase (the time between harvesting of one generation and planting of the next generation) involves burning harvest residues in-situ. However, this practice leads to underutilization of large quantities of plant resources due to combustion loss (Lehmann, 2007) while aggravating soil erosion and air pollution. Although nitrogen (N) and P fertilizers or lime can be added to rectify soil nutrient deficiencies, this practice is not sustainable due to the high economic cost, reduction in downstream water quality due to nutrient runoff and increased emission of soil greenhouse gases (Mitchell et al., 2016).

Sustainable P management is, thus, of great importance to maintain plantation productivity while

minimizing negative environmental impact. Biochar addition to plantation soils may assist with meeting this aim as there is strong evidence that it increases the growth of woody plants (Thomas and Gale, 2015). Biochar is a predominantly stable, recalcitrant organic carbon-rich material produced by pyrolysis of biomass such as crop straw, sawdust, animal manure, wood, and sludge, at temperatures ranging between 300 and 1000 °C (Verheijen et al., 2010). Biochar can be a direct source of P to soil as biomass xylem tissue releases phosphate during carbonization (de la Rosa et al., 2014). In acidic soil, the availability of P is mainly determined by its interaction with Al, Fe, and Ca. The addition of biochar can increase the availability of P in acidic soil due to the increase in soil pH and Ca content, resulting in decreased soil phosphate sorption capacity by Fe and Al hydrous oxides and Al^{3+} (Chintala et al., 2014; Bornø et al., 2018; Hong et al., 2018). Biochar offers several other benefits, including increased bioavailability of other essential plant nutrients (Haefele et al., 2011), enhanced soil water and nutrient retention, and improved soil structure and drainage due to its high porosity (Karhua et al., 2011). Potential detrimental consequences of biochar addition to soil have also been investigated. The environmental risk from metals, metalloids and PAHs contained in biochar has been assessed to be low (Freddo et al., 2012). However, persistent free radicals in biochars have been shown to inhibit seedling germination and growth in soil-free laboratory conditions, although it is possible that these free radicals may be inactivated by natural organic matter and clay in soils (Liao et al., 2014).

Additionally, biochar seems to have beneficial effects in increasing the activity of soil bacteria and fungi and altering the soil microbial community, which could increase soil nutrient availability and carbon storage (Anderson et al., 2011; Mitchell et al., 2016). Soil microbes, such as bacteria, have the ability to convert bound P into available P, making it easier for plants to uptake (Anderson et al., 2011). Significant alterations of the microbial community structure in biochar-amended soil have

been observed (Lehmann et al., 2011; Muhammad et al., 2016; Huang et al., 2017; Yao et al., 2017a,b; Halmi et al., 2018), which can promote important processes such as P solubilization and P mineralization (Schmalenberger and Fox, 2016). This can result in increased soil available P content through increased activity of soil phosphatase and enhanced microbial dissolution of inorganic fixed P and mineralization of organic P (Gul and Whalen, 2016; Zhu et al., 2018; Xu et al., 2019). Nevertheless, some studies have reported no change in soil microbial community structure after biochar addition (e.g. Yu et al., 2018), whilst in others the observed change has been associated with accelerated soil organic N turnover which might induce N limitation for plants (Tian et al., 2016).

Despite increasing understanding of the potential positive effects of biochar amendment in agricultural systems (Atkinson et al., 2010; El-Naggar et al., 2019), the direct and indirect effects of biochar on P dynamics are less well characterized, particularly in forest soils. As biochar properties vary depending on production technology, pyrolysis temperature, and feedstock type (Gul et al., 2015), biochars can have varying effects on soil chemical and biological properties (Bornø et al., 2018). Furthermore, the amount of biochar applied controls the potential beneficial effects of amendment (Noyce et al., 2015). A better understanding of the potential effects of biochar with different properties on P dynamics in forest soil is needed to inform future forest management practices with respect to biochar amendment. Thus, the objective of this study was to examine how biochars produced from the harvest residue of *C. lanceolata* (leaves and woodchips) at different pyrolysis temperatures affect the P dynamics in second generation *C. lanceolata* plantation soil. We hypothesized that biochar addition to forest soil enhances soil P availability with the effect increasing with application rate, and depending on biochar properties, by: (1) increasing soil pH and decreasing the activity or availability of cations (i.e., Al^{3+}, Fe^{3+} and Ca^{2+}) that decrease P sorption or increase P desorption in soil; (2) contributing highly soluble P from the biochar itself that will directly increase soil available P; and

(3) increasing activities of phosphatase and the soil bacterial community, which play an important role in transforming inaccessible organic and inorganic P to available P. To test these hypotheses, an incubation experiment, involving two biochar types (leaf biochar and woodchip biochar), produced at two pyrolysis temperatures (300 and 600 °C) and applied at two rates (1% and 3% *w/w*) was conducted for 80 days to determine soil total P, available P, inorganic and organic P pools, soil phosphatase activity and the composition and diversity of the soil bacterial community.

2. Materials and Methods

2.1. Soil sampling

Soil for the incubation experiment was collected from a second generation *C. lanceolata* plantation at Xinkou Teaching Forest of Fujian Agricultural and Forestry University in Sanming, Fujian province, China (117°27′–118°14′E and 26°07′–27°13′N). The soil was classified as mountain acidic red loam soil based on the Chinese soil classification system, which is equivalent to humic planosols in the FAO system. Soil samples (~50 kg total) were taken from the surface soil (0–20 cm, which contains more than 60% of the fine roots of *C. lanceolata* (Huang et al., 2016) in a 20 m x 20 m plot with an S-shape sampling scheme. The soil samples were combined together, homogenized and screened using a 2 mm sieve, and then air-dried (12 h) and stored at 4 °C before starting the incubation experiment 2 weeks later. The nutrient contents and pH of the prepared *C. lanceolate* forest soil were determined as described below.

2.2. Biochar production and characterization

Woodchips and leaves after harvesting of *C. lanceolata* at the soil sampling site were used as separate feedstocks for preparation of biochar. In the laboratory, leaves were washed with deionized water to

144 remove any residual surface soil, dried at 80 °C, crushed using a mechanical pulverizer (Y-800G,
145 Xuman, China) and then screened using a 1 mm sieve. Woodchips were created in the laboratory from
146 branches following the same procedure as for leaves and then pulverized and screened. The prepared
147 material was heated at 300 °C at 600 °C in a muffle furnace with a heat increase of 20 °C min^{-1} for 4
148 hours and then left to cool overnight. The Fe, Al and Ca content of biochar and blank digests were
149 determined by ICP-OES (Optima 8000, PerkinElmer, USA) of 0.15 g sub-samples digested with
150 HNO_3 at 120 °C for 24 h (open vessels on a hot plate). The instrument was calibrated with 5 standards
151 and a blank (Millipore water) and a standard was analyzed for quality control every 25 samples. The
152 C and N content were measured by elemental analyzer (vario MAX, Elementar, Germany). Biochar
153 pH was measured with a LL-Ecotrode Plus electrode (Metrohm, Switzerland) in a 1:2.5
154 (biochar:deionized water) suspension. The ash content was determined by weight loss after heating
155 the biochars at 750 °C for 4 h in a muffle furnace (Yuan et al. 2011). Biochar dissolved organic carbon
156 (DOC) content was determined by shaking the biochar with 2 M KCl (1:25, *w/v*) for 1 h, filtering
157 through a 0.45 μm PES membrane filter (Jinteng, Tianjin Jinteng Experiment Equipment Co., Ltd.,
158 China), and measuring the DOC concentration with a TOC-V_{CPH} (Shimadzu, Japan). Available and
159 total P contents of the biochars was determined using the procedures described in 2.4.
160
161 *2.3. Soil incubation experiment*
162 To examine the effects of biochar addition to forest soil on P availability, an incubation
163 experiment involving the two biochar types (leaf and woodchip biochars) and two application rates
164 (1% and 3% *w/w*) was conducted for 80 days. The factorial experimental design involving eight
165 biochar treatments and the unamended soil control is shown in Table 1. The experiment had three
166 timesteps in which samples from each treatment were taken after 7, 40 and 80 days of incubation for
167 analysis of chemical and biological properties. There were four replicates for each treatment and
168 timestep combination, giving a total of 108 incubated soil samples. The air-dry soil (50 g dry weight
169 equivalent) and the relevant biochar type and amount were well-mixed individually for each replicate

before adding deionized water to 60% field capacity. Then the biochar-amended samples and control soil samples were placed in separate glass boxes, sealed, and incubated at 20 °C in the dark. The soil moisture was maintained at 60% field capacity by weighing and adding deionized water every 2-3 days.

Table 1. The control and different biochar treatments applied to the forest soil. Biochar additions are % *w/w*.

Treatment abbreviation	Treatment description
CK	unamended soil control
BW3001	1% 300 °C woodchip biochar
BW3003	3% 300 °C woodchip biochar
BW6001	1% 600 °C woodchip biochar
BW6003	3% 600 °C woodchip biochar
BL3001	1% 300 °C leaf biochar
BL3003	3% 300 °C leaf biochar
BL6001	1% 600 °C leaf biochar
BL6003	3% 600 °C leaf biochar

2.4. Phosphorus characterization in soil/biochar samples

Available P was extracted with ammonium fluoride (NH_4F) and hydrochloric acid (HCl) (Liu et al., 2017). Briefly, 50 mL of a mixture of 0.03 mol L^{-1} NH_4F and 0.025 mol L^{-1} HCl were added to 5.0 g sample, and the mixture was agitated on an oscillating shaker (SPH-2102C, Shiping, China) for 5 minutes, before filtration through P-free filter paper (Whatman, China) to separate the solid and

liquid. Total P in soil/biochar samples was determined on digests prepared as follows: 10 mL H_2SO was added to 0.25 g samples, which were left overnight. After adding 1 mL $HClO_4$, the samples were digested on a hot plate at 300 °C for 2 h (Lu, 1999). Phosphorus concentrations in all extractions were measured using the molybdenum blue method with an ultraviolet-visible spectrophotometer (T6, Puxi, China) at 700 nm (Lu, 1999). The instrument was calibrated with 5 standards and a blank (Millipore water) and a standard was analyzed for quality control every 25 samples. Blank extractions were also conducted for available and total P and the values subtracted from the sample extractions.

Soil samples taken at each incubation timestep were air dried, pulverized and passed through a 0.149 mm sieve. Different forms of phosphorus were determined by sequential extraction of 1 g prepared soil as follows (Lei et al., 2017; Li et al., 2017): (1) Ca_2-P: 50 mL of 0.25 mol L^{-1} $NaHCO_3$ (pH 7.5) added and shaken for 1 h; (2) Al-P: 50 mL of 0.5 mol L^{-1} NH_4F (pH 8.5) added and shaken for 1 h; (3) Fe-P: 50 mL of 0.1 mol L^{-1} NaOH added and shaken for 4 h; (4) O-Al-P: 50 mL of 1 mol L^{-1} NaOH added and heated in a water bath at 85 °C for 1 h; (5) O-Fe-P: 50 mL of 0.5 mol L^{-1} $Na_2S_2O_4$-$Na_3C_6H_5O_7$ added and heated in a water bath at 80 °C for 10 minutes; (6) Ca_{10}-P: 50 mL of 0.25 mol L^{-1} H_2SO_4 added and shaken for 1 h. Mixtures were shaken on an oscillating shaker (SPH-2102C, Shiping, China), then suspensions were centrifuged (5810 R, Eppendorf, Germany) at 3200 g for 5 minutes between each extraction step to separate the supernatant for analysis and the residue. Blank extractions were also conducted for each step and the values subtracted from the sample extractions.

2.5. Determination of soil enzyme activity

The activities of acid and alkaline phosphatase were determined for soil samples at each incubation timestep following the method described by Jin et al. (2016). Specifically, for phosphomonoesterase activities analysis, 1 g moist soil was mixed with 4 mL of universal buffer (pH 6.5 for acid phosphomonoesterase and pH 11 for alkaline phosphomonoesterase) and 1 mL of p-nitrophenyl phosphate, gently shaken and incubated for 1 h at 37 °C. The reaction was terminated by

adding 1 mL 0.5 M $CaCl_2$ and 4 mL 0.5 M NaOH solution. After filtering through Whatman No. 40 filter paper, the absorbance of the solution was measured at 410 nm with a spectrophotometer (Puxi/T6, China).

2.6. Determination of soil bacterial diversity and composition

The diversity and composition of the soil bacterial community were determined at the end of the incubation period (80 days) in the control soil and 3% biochar treatments (0.5 g fresh soil) where the greatest changes were expected with the higher biochar application rate. Three of the four replicate samples in each incubation treatment were analyzed.

High-throughput sequencing of 16S rDNA PCR products was conducted by Guangzhou Genedenovo Biotechnology Co., Ltd., China. DNA was extracted from 0.25 g field-moist soil using the FastDNA® SPIN Kit for Soil (Bio 101, Vista, CA, USA) according to the manufacturer's instructions, and then stored at -70 °C for subsequent analysis. After genomic DNA was extracted from the samples, the V3 + V4 region of 16S rDNA was amplified with barcode-specific primers. The primer sequences were as follows: 341F: 5'- CCTACGGGNGGCWGCAG -3' and 806R: 5'-GGACTACHVGGGTATCTAAT-3'. The amplifications were conducted in 50 μL reactions consisting of 5 μL KOD buffer (10 x dose concentration), 5 μL 2.5 mmol L^{-1} dNTPs, 1.5 μL primer (5 μmol L^{-1}), 1 μL KOD polymerase, and 100 ng template DNA. The amplification conditions were: pre-denaturing at 95 °C for 2 min, denaturing at 98 °C for 10 s, annealing at 62 °C for 30 s, and extension at 68 °C for 10 min, repeated for 27 cycles, followed by a final extension at 68 °C for another 10 min.

The amplified PCR products were resolved by agarose gel electrophoresis, using 2 % agarose gel stained with ethidium bromide (0.5 μg mL^{-1}), and visualized and documented by fluorimetry (Quanti Fluor™, Promega, USA). The PCR mixture contained 25.0 μL Quanti Fluor™ (1.25 U DNA polymerase, 4 mmol L^{-1} Mg^{2+}, 0.4 mmol L^{-1} dNTP mixture), 1.0 μL 20 mmol L^{-1} forward primer, 1.0 μl 20 μmol L^{-1} reverse primer, 1.0 μL template DNA (about 100 ng), and 22.0 μL nuclease-free water

to a final volume of 50 μL. The samples were passed through illustra MicroSpin S-300 HR Columns (GE Healthcare Life Sciences, USA) for PCR purification. For each sample, an 8-digit barcode sequence was added to the 5' end of the forward and reverse primers (Guangzhou Genedenovo Biotechnology Co., Ltd., China).

A sequencing library was constructed following the protocols for Illumina platforms (Caporaso et al., 2010), and the amplified products were sequenced using the PE250 mode of Hiseq 2500 (Edgar et al., 2011). The reads with bases having a quality score below 20 were discarded because it was difficult to interpret the reads below this threshold. Then, tags were intercepted and filtered by length (Haas et al., 2011). Finally, the tag sequences were compared with the Gold database r20110519 using the UCHIME algorithm to detect and remove chimeric sequences (Wang et al., 2007; Edgar, 2013) and obtain the final effective tags. The effective tag sequences of all samples were clustered to form operational taxonomic units (OTUs) using Uparseusearch v9.2.64. To construct OTUs, representative sequences were selected based on the 97% similarity threshold, including the tag sequences with the highest abundance of OTUs. The set of representative sequences was annotated by RDP Classifier (version 2.2) with a confidence threshold of approximately 0.8-1. The SILVA taxonomic library (http://www.arb-silva.de) was used to assign taxonomy to the sequences (Yilmaz et al., 2014).

Venn diagrams of shared OTUs (97% similarity) between the control and biochar-amended soils (Fig. S1) revealed a total of 2432 separate OTUs after 80 days incubation. Sequences were randomly selected based on the relative ratio of known OTUs in obtained sequences, and the rarefaction curve was constructed by plotting the number of OTUs against the number of tags sampled. The gentle slope of the curves (Figs. S2 and S3) indicated that the depth of sequencing had covered all species in the samples.

2.7. Statistical analysis

All statistical analyses were conducted with SPSS v22. Before performing the statistical analyses,

data were tested for deviations from normality and homogeneity of variance. One-way ANOVA and comparison of means with Tukey's honestly significant difference post-hoc test ($p < 0.05$) were used to assess any significant differences between the characteristics of the four biochars used in the experiment and between the bacterial community diversity indices of the control and 3% biochar amended soils after 80 days incubation. The effects of biochar addition on available P, total P, different P fractions and phosphatase enzyme activities in forest soil were analyzed by repeated measures ANOVA. When the homogeneity of variance assumption was violated, according to Mauchly's test of sphericity, the degrees of freedom for testing the significance of the within-subject factors were adjusted using the Huynh–Feldt correction factor. Tukey's honestly significant difference test was employed for post-hoc comparisons ($p < 0.05$). For bacterial diversity, QIIME 1.7.0 (Caporaso et al., 2010) was used to calculate the Chao1, ACE, Shannon, and Simpson indices.

Principal Component Analysis (PCA) was used to compare soil bacterial community structure between the different treatments. The evolution distances between microbial communities from each sample were calculated using the tayc coefficient and represented as an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering tree describing the dissimilarity (1 - similarity) between multiple samples. To compare the membership and structure of communities in different samples, heat maps were generated with the top 20 OTUs using Mothur.

3. Results

3.1. Biochar properties

The biochars produced using leaves and woodchips at different pyrolysis temperatures had significantly different pHs and chemical composition (Table 2). Biochars produced from leaves had higher pH, ash content, available P, and total P, N, and Ca content and lower C:N ratio compared to

the woodchip biochars. For both biochar feedstocks, increasing pyrolysis temperature resulted in higher pH, ash content and DOC, available P and total Ca content at 600 °C than at 300 °C. While the total C and Fe content did not vary between leaf and woodchip biochars, the Al content was significantly higher for the woodchip biochar (>1900 mg kg^{-1}) than the leaf biochar (<1100 mg kg^{-1}).

3.2. Total and available soil phosphorus contents during the incubation experiment

Soil total P and available P content varied significantly among treatments, incubation time and their interaction (Table 3). The application of biochar from *C. lanceolata* leaves resulted in significantly higher total and available P contents in soil compared to the control and woodchip biochar treatments, with the increase being most pronounced at higher application rates and pyrolysis temperature (Figs. 1 and S4).

The BL3003 and BL6003 treatments increased mean soil total P by 85.4 mg kg^{-1} (28%) and 211 mg kg^{-1} (70%), respectively, and mean available P by 5.86 mg kg^{-1} (45%) and 20.9 mg kg^{-1} (161%), respectively, after 80 days of incubation compared with the control. Soil available P content decreased over time during the experiment within each treatment (Figs. 1 and S4D-F). After 80 days of incubation the mean available P content in the BW3003 and BW6003 treatments was 62% (8.03 mg kg^{-1}) and 60% (7.82 mg kg^{-1}), respectively, that of the control (13.0 mg kg^{-1}), while the total P content of the woodchip biochar treatments varied little with the duration of the study compared with the control (Figs. 1 and S4A-C).

303 **Table 2.** Mean (± SE, n=4) chemical compositions of the soil and biochar. Means with different letters across a row indicate significant differences ($p <$

304 0.05) between the biochars.

305

Properties	BW300	BW600	BL300	BL600	Soil
pH (%)	4.05 ± 0.01a	7.96 ± 0.01c	7.33 ± 0.02b	10.4 ± 0.01d	4.34 ± 0.06
Ash (%)	1.00 ± 0.2a	2.70 ± 0.9a	9.90 ± 0.5b	22.2 ± 0.2c	-
DOC* (g kg^{-1})	1.26 ± 0.1c	0.30 ± 0.03a	2.44 ± 0.26d	0.96 ± 0.04b	0.51 ± 0.02
Total P (g kg^{-1})	0.12 ± 0.02a	0.14 ± 0.03a	0.82 ± 0.05b	1.51 ± 0.11c	0.32 ± 0.73
Available P (g kg^{-1})	0.23 ± 0.00a	0.62 ± 0.05b	2.57 ± 0.16c	3.6 ± 0.20d	0.014 ± 0.00
Total C (%)	59.2 ± 0.07a	67.7 ± 4.2a	56.3 ± 3.3a	59.2 ± 1.0a	1.63 ± 0.12
Total N (%)	0.39 ± 0.02a	0.35 ± 0.04a	1.57 ± 0.01d	1.28 ± 0.01c	0.18 ± 0.02
C:N ratio	151.8±9.2c	193.4±11.5d	35.6±3.9a	46.3±8.5b	9.1±1.5
Ca (g kg^{-1})	2.31 ± 0.18a	7.62 ± 1.34a	33.50 ± 0.88b	61.58 ± 0.44c	7.8 ± 0.09
Fe (g kg^{-1})	2.49 ± 0.54a	2.74 ±0. 46a	2.21 ± 0.14a	1.97 ± 0.18a	672 ± 41.3
Al (g kg^{-1})	1.95 ± 0.17b	1.99 ± 0.23b	1.07 ± 0.15a	0.91 ± 0.13a	2.24 ± 0.27

306 * Dissolved organic carbon

307 **Table 3.** Summary of repeated measures ANOVA for testing the significance of the between-
308 subject (treatment) and within-subject (time) effects on soil total P, available P, and different P
309 fractions, as well as soil acid phosphatase (ACP) and alkaline phosphatase (ALP) activities.

310

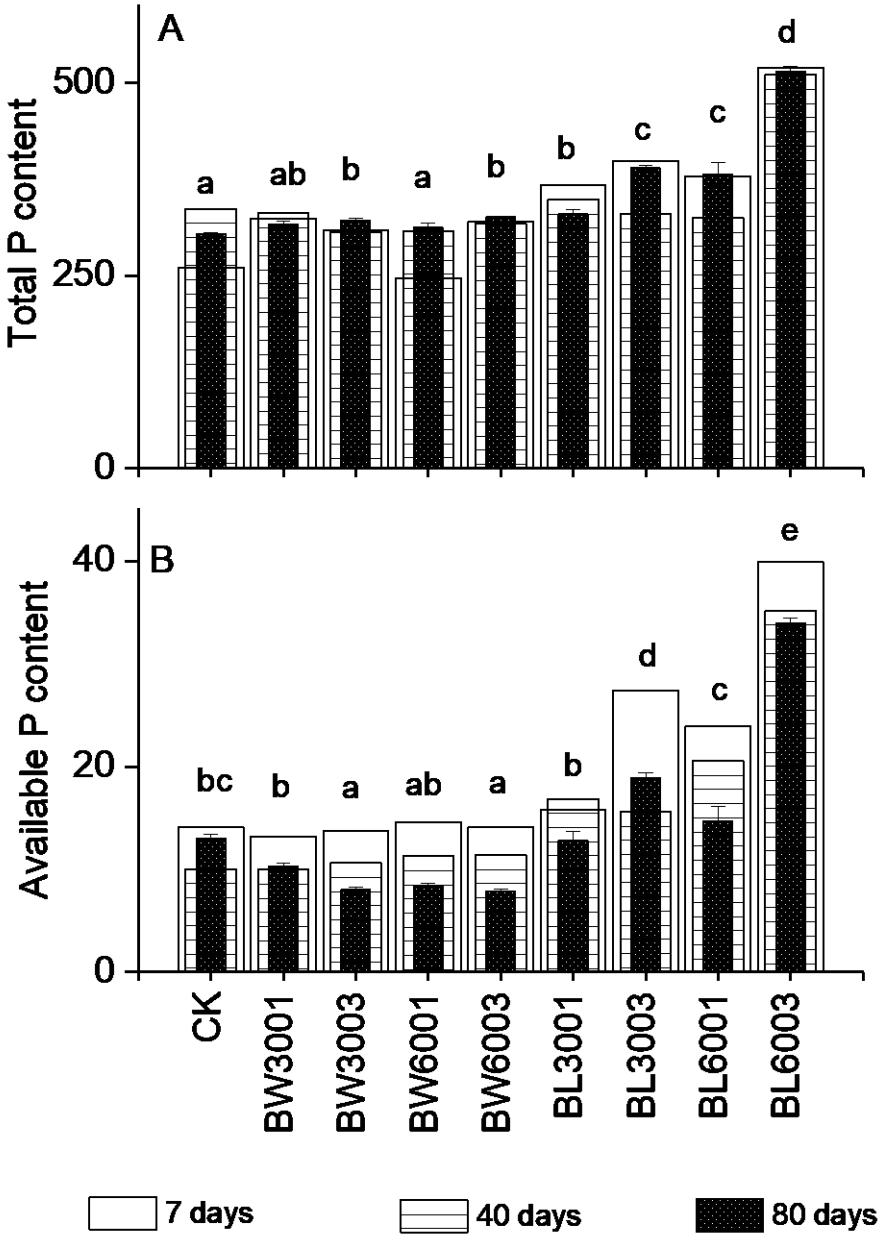
Variables	Between-subject factor (d.f. = 8)		Within-subject factor (d.f. = 2)		Interaction (d.f. = 16)	
	F	*p*	F	*p*	F	*p*
Total P	82.9	<0.001	9	<0.001	7.5	<0.001
Available P	169.6	<0.001	84.3	<0.001	5.6	<0.001
Al-P	8.3	<0.001	130.9	<0.001	3.7	<0.001
Fe-P	15.9	<0.001	14.9	<0.001	9.7	<0.001
Ca_2-P	22.1	<0.001	134.4	<0.001	8.3	<0.001
Ca_{10}-P	33.2	<0.001	133	<0.001	4	<0.001
O-Al-P	6.7	<0.001	391	<0.001	11.2	<0.001
O-Fe-P	3.6	0.006	104.5	<0.001	0.91	0.559
ACP	27.6	<0.001	121.7	<0.001	3.8	<0.001
ALP	11	<0.001	78.9	<0.001	2.5	0.007

311

312 **Figure 1.** Soil total phosphorus (panel A) and available phosphorus (panel B) contents (mg kg^{-1}, mean
313 ± SE, n=4) after different incubation times following addition of biochar produced from *C. lanceolata*
314 leaves and woodchips to forest soil. The treatment abbreviations are shown in Table 1. Bars with
315 different letter(s) are significantly different among 80 day incubations ($p < 0.05$). Note different y-
316 axis scales.

317

318

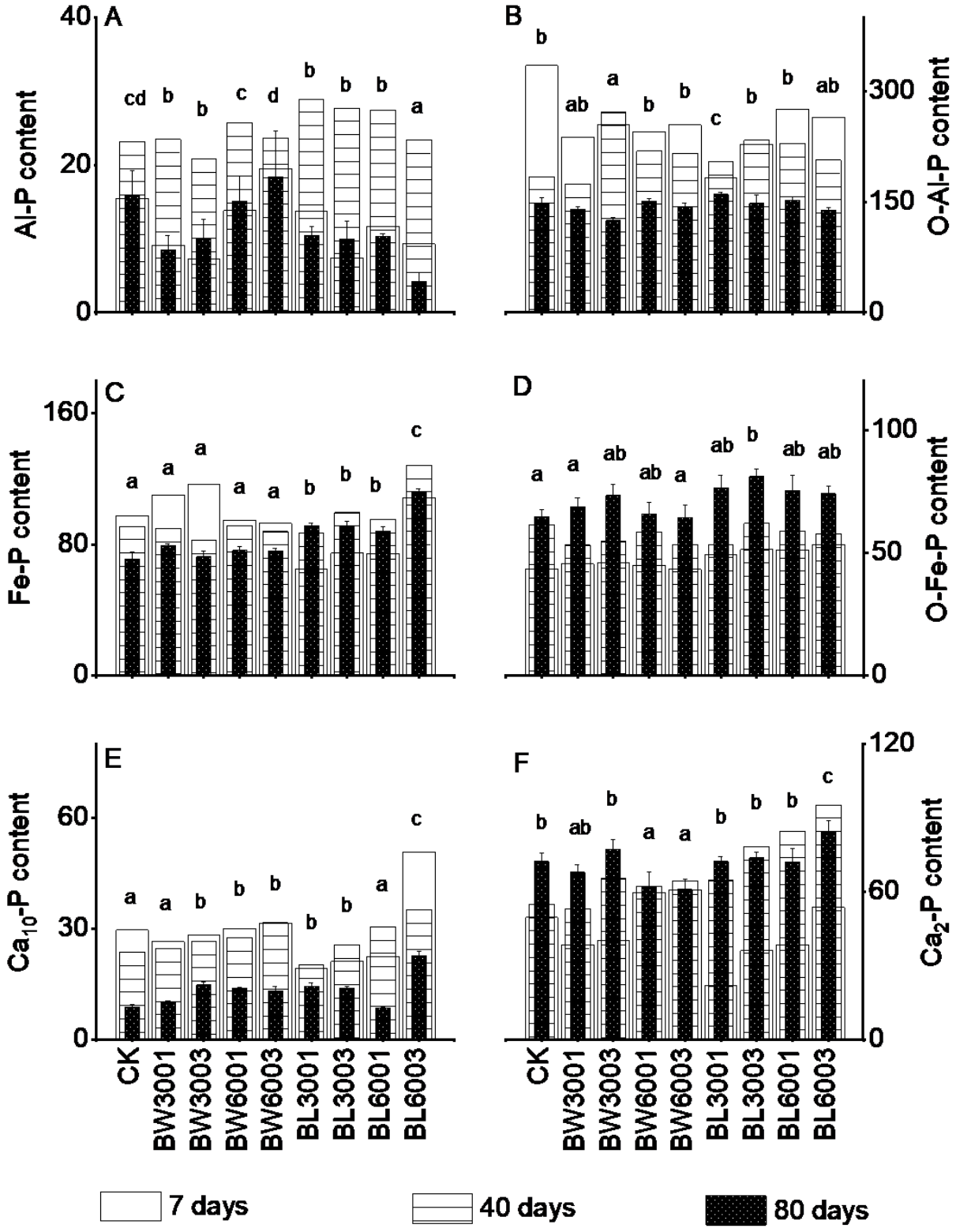


319 *3.3. Dynamics of soil P forms in biochar-amended forest soil*

320 Significant differences were detected among treatments, incubation time and their interaction
321 for all soil P forms, except O-Fe-P for which no significant interaction effect was detected (Table 3,
322 Figs. 2, S5, S6 and S7). In all biochar treatments and the control, soil Al-P content increased between
323 7 and 40 days and then decreased after 80 days incubation (Figs. 2A, S5A, S6A and S7A). At the end
324 of the experiment, soil Al-P content was significantly lower than the control in all the biochar
325 treatments, apart from the 600 °C woodchip biochar treatments. The soil O-Al-P content decreased
326 continuously over time in nearly all treatments (Figs. S5B, S6B and S7B). After 80 days, only the
327 BL3001 and BW3003 treatments had significantly lower soil O-Al-P content than the control (Fig.
328 2B). The mean soil Fe-P content decreased over time for the control and woodchip biochar treatments
329 and was not significantly different after 80 days of incubation (Figs. 2C, S5C, S6C and S7C). In
330 contrast, the soil Fe-P content in the leaf biochar treatments was significantly higher than in the
331 control after 80 days incubation. Soil O-Fe-P content increased continuously over time during the
332 experiment (Figs. 2D, S5D, S6D and S7D). The only significant difference in soil O-Fe-P content
333 between the treatments after 80 days incubation, was a small but significant increase in the BL3003
334 treatment soil compared with the control and the BW3001 and BW6003 treatments. The soil Ca_{10}-P
335 content decreased over time for most of the treatments (Figs. 2E, S5E, S6E and S7E). After 80 days
336 incubation, soil Ca_{10}-P content was significantly higher in all the biochar treatments compared with
337 the control, apart from the BW3001 and BL6001 treatments. Mean soil Ca_2-P content generally
338 increased between 7 days and 40 days during the experiment and then stabilized in most treatments
339 (Figs. 2F, S5F, S6F and S7F). After 80 days incubation, compared with the control, the BL6003
340 treatment was the only treatment with a significantly higher Ca_2-P content, whilst the BW6001 and
341 BW6003 treatments had a significantly lower Ca_2-P content.

342

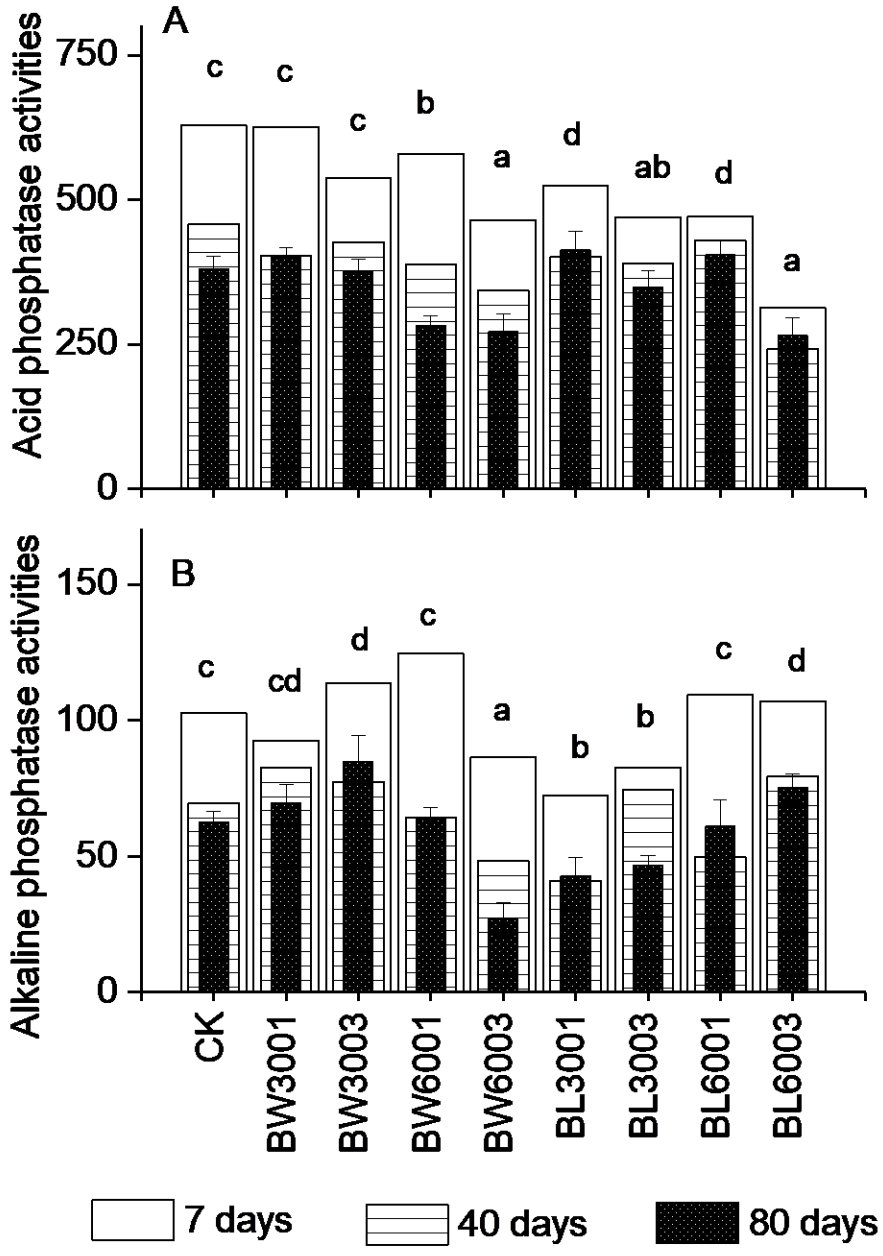
Figure 2. Soil P fractions (panel A is Al-P, panel B is O-Al-P, panel C is Fe-P, panel D is O-Fe-P, panel E is Ca_{10}-P, and panel F is Ca_2-P) contents (mg kg^{-1}, mean ± SE, n=4) after different incubation times following addition of biochar produced from *C. lanceolata* leaves and woodchips to forest soil. The treatment abbreviations are shown in Table 1. Bars with different letter(s) are significantly different among 80 day incubations ($p < 0.05$). Note different y-axis scales.



3.4. Effects of biochar application on soil enzyme activities

Acid phosphatase showed higher activities than alkaline phosphatase in the control and biochar-amended soil samples throughout the experiment. Both soil acid and alkaline phosphatase activities varied significantly among treatments, incubation time and their interaction (Table 3, Figs. 3 and S8). Acid phosphatase activities decreased after 40 and 80 days of incubation compared to 7 days of incubation in almost all treatments (Figs. 3A and S8A-C). After 80 days incubation, among treatments, the activity of acid phosphomonoesterase decreased with biochar pyrolysis temperature and application rate, and significantly for the BW6001, BW6003 BL3003 and BL6003 treatments, but was significantly higher in the BL3001 and BL6001 treatments compared to the control (Fig. 3A). The soil alkaline phosphatase activity also declined over time in most of the treatments (Figs. 3B and S8D-F). After 80 days incubation, the activity of this enzyme was significantly reduced in the BW6003, BL3001 and BL3003 treatments, and increased in the BW3003 and BL6003 treatments, compared to the control (Fig. 3B).

Figure 3. Soil acid phosphatase (A) and alkaline phosphatase (B) activities (mg kg^{-1} h^{-1}, mean ± SE, n=4) after different incubation times following addition of biochar produced from *C. lanceolata* leaves and woodchips to forest soil. The treatment abbreviations are shown in Table 1. Bars with different letter(s) are significantly different among 80 day incubations ($p < 0.05$). Note different y-axis scales.



370 *3.5. Diversity and composition of soil bacterial community*

371 After 80 days incubation, the control, BL3003, BL6003, BW3003, and BW6003 treatments
372 contained 1451, 1478, 1375, 1328, and 1339 OTUs, respectively, of which BL3003 accounted for the
373 largest number of OTUs. Compared with the control, the number of unique OTUs was the largest in
374 the BL3003 treatment, at 403 OTUs, indicating that there were more unique bacterial species
375 following this treatment. Also, the number of OTUs shared by the BL3003 treatment and the control
376 was the largest at 1075 (Fig. S1). The alpha diversity indices of soil bacteria across the 3% biochar
377 addition treatments and the control after 80 days incubation are given in Table 4.

378

379 **Table 4.** Omicsmart MiSeq sequencing bacterial data and bacterial community diversity indices (at
380 97% sequence similarity) based on the 16S rRNA gene after 80 days incubation. The treatment
381 abbreviations are shown in Table 1. Different letters within the same column indicate significant
382 difference between treatments ($p < 0.05$). The treatment abbreviations are shown in Table 1.

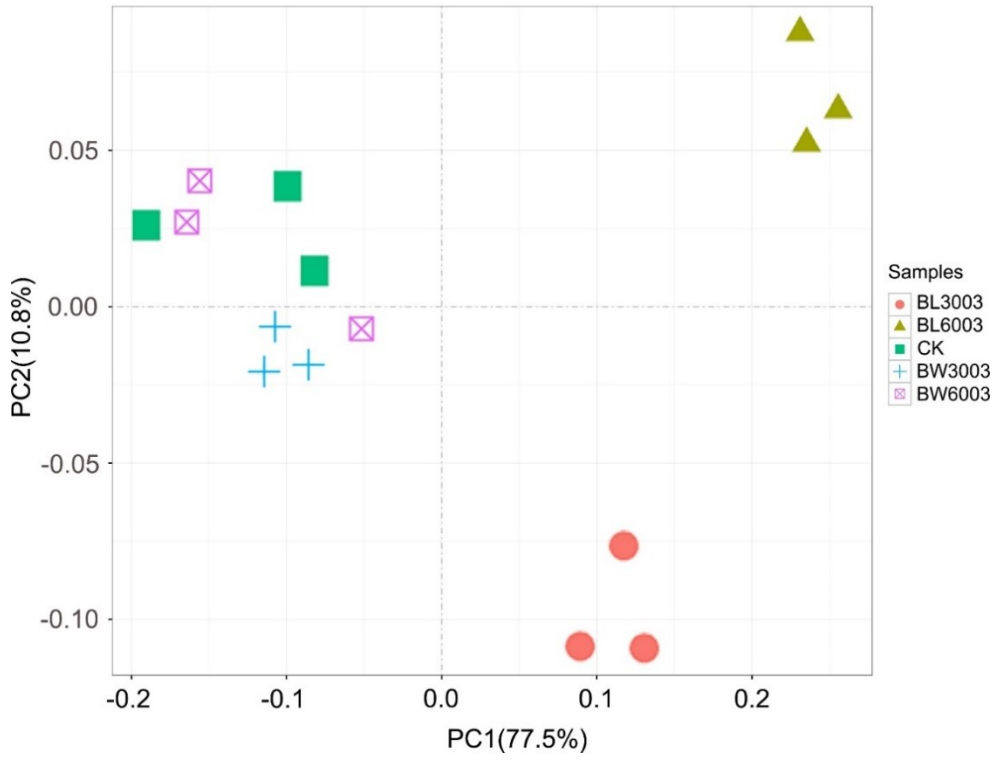
383

Treatment	Chao1	ACE	coverage	Shannon	Simpson	OTUs
CK	1906±193a	1889±117a	0.996	6.973±0.26a	0.967±0.11a	1451±217a
BL3003	2051±164a	1889±115a	0.996	7.337±0.03b	0.986±0.11a	1478±63a
BL6003	1776±43a	1889±116a	0.996	7.177±0.16ab	0.981±0.181a	1357±14a
BW3003	1838±141a	1832±121a	0.996	6.787±0.06a	0.968 ±0.21a	1328±114a
BW6003	1927±93a	1889±114a	0.996	6.733±0.24a	0.963±0.23a	1339±202a

384

385

386 There was no significant difference in the number of OTUs or any of the indices between the
387 treatments, with the exception of the Shannon index. The Shannon index, which is a measure of
388 species richness and evenness, was significantly higher for the BL3003 treatment than the BW3003
389 and BW6003 treatments and the control. The PCA also showed that the application of the biochar
390 produced from leaves caused divergence in the community composition (Fig. 4). The contribution of
391 the first principal component (PC1) was 77.5% and that of the second principal component (PC2)
392 was 10.8%. The treatments with biochar from *C. lanceolata* leaves were clearly distinguished from
393 the woodchip biochar treatments and the control along PC1 (the x-axis), whilst the woodchip biochar
394 and control samples plotted close together. The leaf biochars produced at the two pyrolysis
395 temperatures were also distinguished along PC2 (the y-axis). These results showed that the
396 application of biochar made from different *C. lanceolata* harvest residues resulted in different soil
397 bacterial community structures, with the source of biochar materials having the most significant
398 influence, followed by the pyrolysis temperature.
399
400 **Figure 4.** PCA ordination of soil bacterial community structure in biochar-amended soil (3% *w/w*)
401 and the control after 80 days incubation. The treatment abbreviations are shown in Table 1.
402

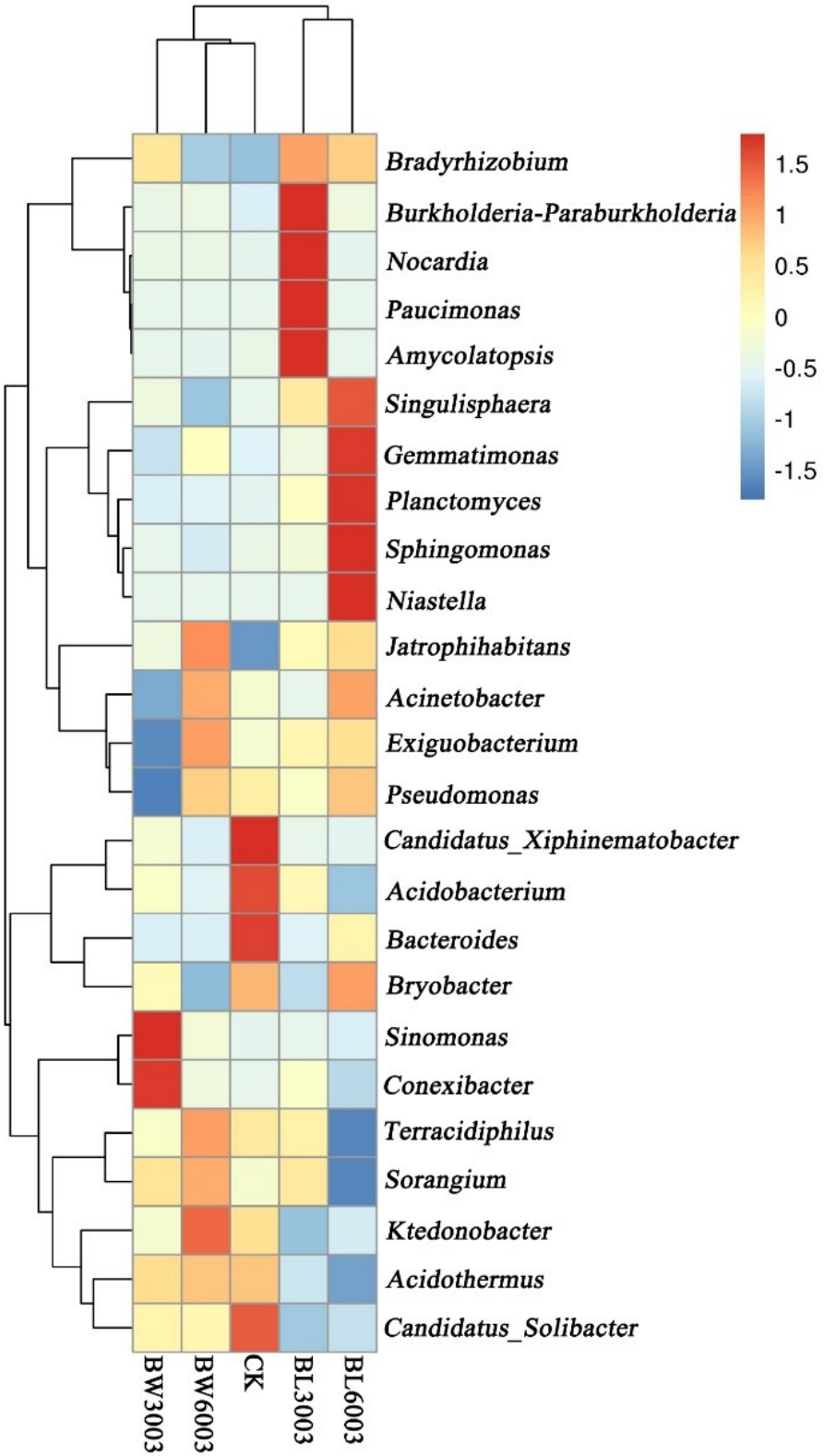


403

404 The top 25 OTUs accounted for 41-52% of the soil bacteria genus relative abundance in the
405 control and biochar treatments, and are detailed in Table S1. The distinctive bacteria genera in the
406 control and each treatment are highlighted in the heat map in Fig. 5. The control had 171 unique
407 OTUs, of which *Candidatus Solibacter, Candidatus Xiphinematobacter, Acidobacterium* and
408 *Bacteroides* were more abundant compared to the biochar treatments, while *Jatrophihabitans* and
409 *Bradyrhizobium* were less abundant. The BW3003 treatment contained 123 unique OTUs, of which
410 *Sinomonas* and *Conexibacter* had the highest relative abundance (6.45% and 1.53%, respectively)
411 compared to other treatments, whereas *Pseudomonas, Exiguobacterium* and *Acinetobacter* were less
412 abundant. Among the 148 unique OTUs identified in the BW6003 treatment, *Ktedonobacter* had the
413 highest relative abundance (11.9%) compared to other treatments, while *Bryobacter, Singulisphaera*
414 and *Bradyrhizobium* were less abundant genera. In the BL3003 treatment, 207 unique OTUs were
415 found of which *Burkholderia-Paraburkholderia, Nocardia, Paucimonas* and *Amycolatopsis* were the
416 most abundant (6.88%, 1.15%, 1.91%, 1.12%, respectively) compared to other treatments, whereas
417 *Ktedonobacter* and *Candidatus Solibacter* were less abundant. The BL6003 treatment contained 238
418 unique OTUs, of which the most abundant were *Gemmatimonas, Planctomyces*, *Sphingomonasi* and
419 *Niastella* (0.79%, 6.38%, 4.87%, 1.73%, respectively) compared to other treatments, while
420 *Terracidiphilus, Sorangium* and *Acidothermus* were less abundant.

421

Figure 5. z-score hierarchical clustering and heat map of soil bacteria genus abundance in the top 25 OTUs in biochar-amended soil (3% *w/w*) and the control after 80 days incubation. Each column in the heat map represents a sample and each row represents a classification level. The color scale indicates the gene species abundance expressed as standard deviations from the mean (the z-score), with red for high abundance and blue for low abundance. The treatment abbreviations are shown in Table 1.

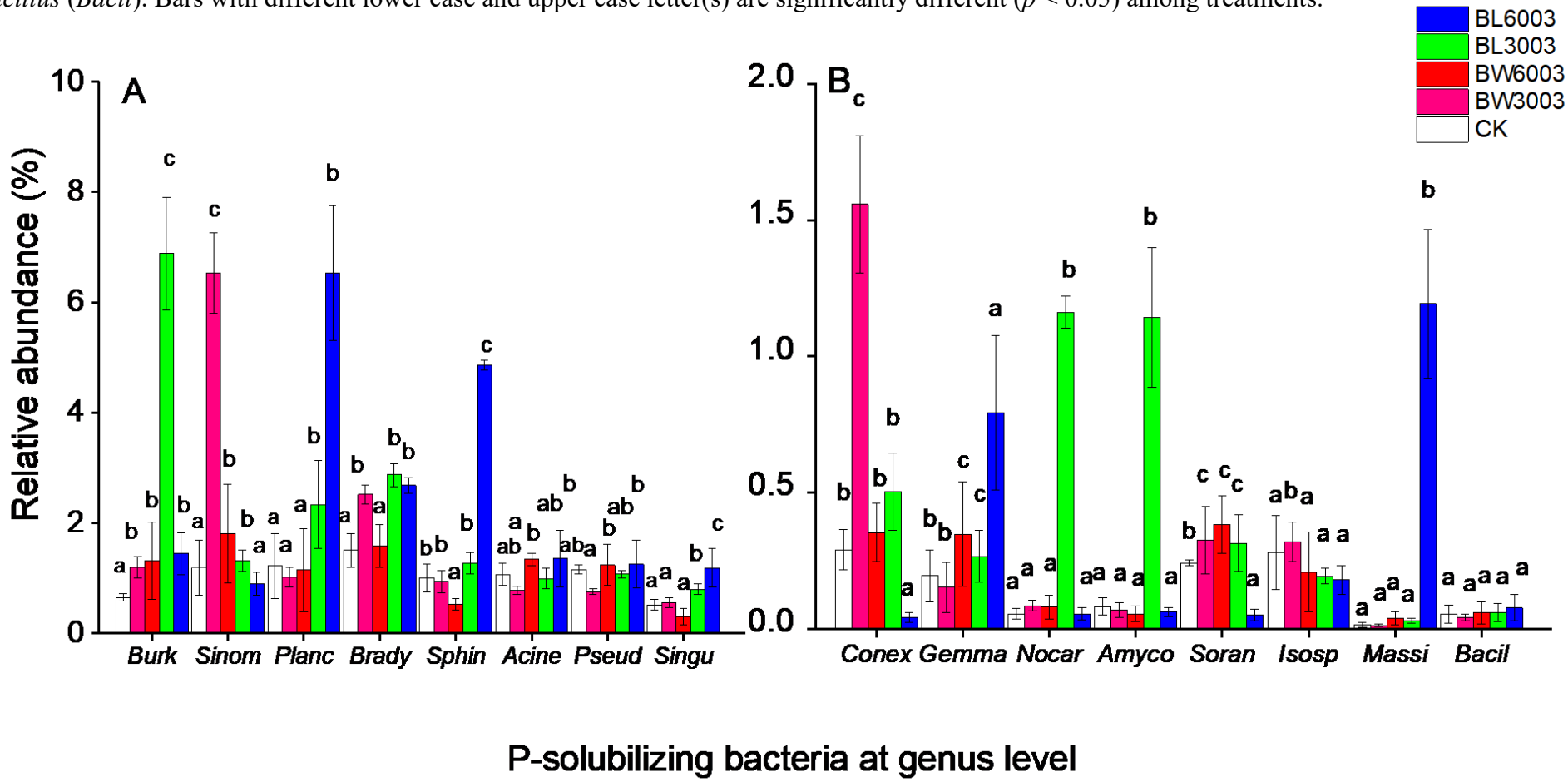


The abundance of several soil P-solubilizing bacteria genera was significantly higher in some of the 3% biochar treatments compared with the control (Fig. 6), particularly for the leaf biochar rather than the woodchip biochar. The abundance of the following P-solubilizing bacteria increased significantly in soil amended with the BL3003 and/or BL6003 treatments compared to the control and the *C. lanceolata* woodchip biochar treatments: *Burkholderia-Paraburkholderia*, *Planctomyces*, *Sphingomonas*, *Singulisphaera*, *Gemmatimonas*, *Nocardia*, *Amycolatopsis*, *Massilia*. The relative abundance of *Burkholderia-Paraburkholderia* increased significantly by 85%, 102%, 959%, and 123% in the BW3003, BW6003, BL3003, and BL6003 treatments, respectively, compared with the control. The relative abundance of *Planctomyces* increased by 91% and 436% in the BL3003 and BL6003 treatments, respectively, compared to the control.

4. Discussion

The results demonstrate that addition of biochar to second rotation *C. lanceolata* plantation soil maintains higher total and available P contents, depending on the feedstock, pyrolysis temperature and the application rate. The main explanations for the changes in soil available and total P content after biochar addition are: 1) the direct addition of P in the biochar, and 2) the indirect effect of biochar addition in altering soil factors which affect total P content and availability, such as soil pH, the content and activity of soil Al^{3+}, Fe^{3+}, and Ca^{2+} and dissolved organic carbon (DOC) which affect soil P fixation, and the soil microbial community structure and activity. Evidence for the operation of these mechanisms in the present study is explored below.

Figure 6. The relative abundance (mean ± SE, n=3) of soil phosphorus-solubilizing bacteria in biochar-amended soil (3% *w/w*) and the control after 80 days incubation. The treatment abbreviations are shown in Table 1. Bacteria genus labels are: *Burkholderia-Paraburkholderia* (*Burk*), *Sinomonas* (*Sinom*), *Planctomyces* (*Planc*), *Bradyrhizobium* (*Brady*), *Sphingomonas* (*Sphin*), *Acinetobacter* (*Acine*), *Pseudomonas* (*Pseud*), *Singulisphaera* (*Singu*), *Conexibacter* (*Conex*), *Gemmatimonas* (*Gemma*), *Nocardia* (*Nocar*), *Amycolatopsis* (*Amyco*), *Sorangium* (*Soran*), *Isosphaera* (*Isosp*), *Massilia* (*Massi*), *Bacillus* (*Bacil*). Bars with different lower case and upper case letter(s) are significantly different ($p < 0.05$) among treatments.



Corresponding with the higher available and total P contents of the leaf biochar, the available and total P contents in soil treated with leaf biochar were significantly higher than that treated with woodchip biochar. These differences increased with temperature and application rate at the start of the experiment, although the significance of differences diminished over time. Application of biochars made from rice straw and branches to rice paddy soil showed similar results, where the soil available P increased more with the application of rice straw biochar, which had a higher P content, than the branch biochar (Chao et al., 2015). The P content in leaf biochar was higher than that of woodchip biochar since leaves contain more mineral nutrients and less carbon compared with woody materials in *C. lanceolata* (Ma et al., 2007), resulting in more available P in soil amended with leaf biochar. Furthermore, partially stable P in biochar feedstock may be activated and become soluble after pyrolysis, with pyrolysis temperature determining the element content and surface physical structure of the biochar (Cheng et al., 2006; Gundale and DeLuca, 2006). This influence of pyrolysis temperature on biochar properties was evident in the present study, as total and available P concentrations were higher in each biochar type produced at 600 °C compared to that from the same feedstock pyrolyzed at 300 °C (Table 2).

As well as the direct effects of P addition in biochar-amended soils, biochar addition may alter many soil properties which indirectly affect soil P dynamics, content and availability (Bornø et al., 2018). Soil pH is an important control on P availability as it is related to fixation of P by Al and Fe at pH < 5.5 and by Ca at pH > 7.5. Previous

477 studies have reported that biochar addition may increase soil pH and change the activity
478 or availability of Al^{3+}, Fe^{3+}, and Ca^{2+}, resulting in changed P sorption/desorption in soil
479 (Xu et al., 2014). Most biochars are alkaline because as the pyrolysis temperature
480 increases, surface acidic groups (e.g. carboxyl, hydroxyl and phenolic groups) decrease
481 and surface basic groups (e.g. lactones) increase (Chen et al., 2014). Also, mineral
482 elements such as Na, K, Mg and Ca, are present in the form of oxides or carbonates in
483 the ash (Wu et al., 2019). The biochars examined in this study had pH > 7.3, apart from
484 the woodchip biochar produced at 300°C (pH 4.05), and were added to second rotation
485 forest plantation soil of pH 4.3. In the first half of the incubation experiment, soil pHs
486 in the leaf biochar treatments (pH 4.7-6.1) were significantly higher than the control
487 soil, but after 80 days soil pH was only significantly enhanced (pH 5.5) in the BL6003
488 treatment (Table S2). Thus soil pH is unlikely to explain the higher available soil P
489 contents in the leaf biochar treatments.

490 A further factor in the present study could be increased immobilization of available
491 P in the woodchip biochar treatments due to the higher Al content of the woodchip
492 biochar compared to the leaf biochar, or in the leaf biochar treatments due to the higher
493 Ca content of the leaf biochars (Table 2). Counteracting this effect is that biochar can
494 adsorb ions with which P can precipitate readily in soil, such as Al^{3+}, Fe^{3+}, and Ca^{2+}
495 (Gundale and DeLuca, 2007), or through the formation of chelates between Al^{3+} and
496 Fe^{3+} and organic molecules adsorbed on the surface of biochar (Xu et al., 2014), thus
497 improving soil P availability. The soil Al-P content results after 80 days incubation
498 indicate that increased immobilization of available P by Al^{3+} did not occur in the present

499 study, since none of the biochar treatments have significantly higher Al-P
500 concentrations than the control, and most are significantly lower. Instead, Al^{3+}
501 inactivation due to biochar addition is most probable. Inactivation of Fe^{3+} and Ca^{2+} by
502 biochar was not evident in this study, since soil Fe-P, Ca_{10}-P and Ca_2-P contents in all
503 biochar treatments were the same as the control or significantly higher (Fig. 2C, E- F).
504 Moreover, the Fe addition in the biochar treatments (~2 g kg^{-1}) was negligible compared
505 to the soil background concentration (~670 g kg^{-1}) (Table 2). There is also no clear
506 evidence of increased formation of chelates with Al^{3+} and Fe^{3+} after biochar addition
507 causing enhanced soil available P, because the soil O-Al-P and O-Fe-P concentrations
508 in most biochar treatments are not significantly different from the control (Fig. 2B, D).
509 The higher DOC content in the biochar made from *C. lanceolata* leaves than the
510 woodchip biochar, produced at the same pyrolysis temperature (Table 2), could also
511 explain the higher soil available P content in the leaf biochar treatments. Various
512 mechanisms have been suggested by which biochar-derived dissolved organic matter
513 could inhibit P sorption on different soil components, such as: 1) soil colloids due to
514 competition for sorption sites and electrostatic repulsive forces (Schneider and
515 Haderlein, 2016); 2) goethite, particularly in acidic, highly weathered soils (Schneider
516 and Haderlein, 2016); and 3) Fe and Al oxides, due to the increase of anion exchange
517 capacity or cation activity resulting from organic matter addition, as reported following
518 the addition of manure-derived biochar to soil (Yual et al., 2014). Whilst the mineralogy
519 of the study soil was not determined, goethite has been detected in the same soil type
520 in the neighboring county (Chen et al., 2018) and the acidic and highly-weathered

521 nature of the study soil indicates that the higher DOC inputs from the leaf biochars
522 could help explain the higher soil available P content in these treatments. Although
523 significantly higher soil available P concentrations were maintained in most of the leaf
524 biochar treatments to the end of the 80-day incubation experiment, concentrations
525 decreased over time, probably due to fixation with Ca or chelation with Fe and organic
526 material (see increase in soil O-Fe-P and Ca_2-P contents over time during the
527 experiment, Fig. 2D and F), adsorption to biochar or mineral surfaces, or net
528 immobilization by the microbial biomass (Nguyen and Marschner, 2005; Xu et al.,
529 2019).

530 Soil enzymes serve several important functions. They are intimately involved in
531 the cycling of nutrients, affect fertilizer use efficiency, and, since they reflect soil
532 microbiological activity, they can act as indicators of soil change. The focus of much
533 soil enzyme research has been to develop methodologies for their measurement and to
534 provide an understanding of their origin and the factors that affect their activity in soil.
535 Comparing enzyme activities between studies can be difficult due to differences in the
536 methodologies used (Peoples and Koide, 2012). The contribution of phosphatase
537 enzymes in increasing soil P availability was minor in the present study. Activities of
538 acid and alkaline phosphomonoesterase decreased significantly in some biochar
539 treatments compared to the control after 80 days incubation with biochar, whilst in
540 others there was an increase in activities or no significant difference between the
541 treatments and the control. These findings of the variable effects of biochar addition on
542 soil phosphatase activities are supported by other studies. Biochar addition to soils has

been reported to increase (Bera et al., 2016; Marzooqi and Yousef, 2017), have no effect (Zhang et al., 2017) or reduce (Foster et al., 2016) phosphatase activity. The lower activities of phosphomonoesterase following biochar amendment of soil has been attributed to several mechanisms (Foster et al. 2016), including: sorption or blockage of the enzyme by biochar, lack of soil liming effect due to biochar addition, and increased soil available P resulting in decreased phosphatase activity. The first two of these explanations are more probable in the present study, since significant differences in soil available P compared with the control did not occur in all of the biochar treatments with reduced enzyme activity.

This study showed that the addition of biochar derived from *C. lanceolata* leaves increased the soil bacterial community diversity. Changes in soil properties after biochar application have been shown to alter the structure of soil bacterial communities (Kolton et al., 2011; Chen et al., 2015; Yao et al., 2017a). Previous studies suggest that, because of its physical properties, such as high nanoporosity and large specific surface area, biochar addition can improve soil bacteria and fungi growth by increasing the overall soil aeration and water retention, and by the biochar itself providing habitat for bacteria and fungi to escape from predators and to live and grow (Quilliam et al., 2013; Yao et al., 2017b; Dai et al., 2018; McCormack., 2019; Zheng et al., 2019). It is hypothesized that, of the biochars used in this study, the leaf-based biochar has characteristics more favorable for enhancing the soil bacterial community (such as larger specific surface area, although not measured) compared to the woodchip biochar. Changes in soil chemical properties, notably soil pH and nutrient content and

565 availability, caused by biochar application can also alter the bacterial community
566 structure (Rousk et al., 2010; Yao et al., 2017a; Simarani et al., 2018). The increased
567 soil pH (at least for the first 40 days) and P content and availability after the addition of
568 biochar prepared from *C. lanceolata* leaves might stimulate the growth and
569 reproduction of soil bacteria, thereby changing the soil bacterial community structure.
570 The results are consistent with previous studies, which demonstrated a larger number
571 of 16S rRNA gene copies (Chen et al., 2015), and increased microorganism total
572 phospholipid fatty acids (Muhammad et al., 2016) and bacterial diversity (Yao et al.,
573 2017a) in biochar-amended compared to unamended control soil samples.

574 P-solubilizing bacteria have been shown to enhance the solubilization of P
575 compounds with limited solubility through the release of organic acids and phosphatase
576 enzymes (Alori et al., 2017; Yao et al., 2017a). In the current study, the abundance of
577 some P-solubilizing bacteria increased significantly in soil amended with biochar
578 derived from *C. lanceolata* leaves. Increased abundance of inorganic phosphate-
579 solubilizing bacterial communities has also been reported following soil amendment
580 with straw biochar (Zheng et al., 2019), and the application of citrus wood biochar to
581 soil was shown to increase the root-associated bacterial populations affiliated with the
582 phyla *Actinobacteria, Bacteroidetes, Firmicutes*, and *Proteobacteria*, which benefit
583 plant growth (Kolton et al., 2011). However, the abundance of genes associated with
584 soil phosphatase synthesis was found to be unaltered 3 months following amendment
585 of agricultural field plots with wood biochar, even though the soil P availability had
586 increased (Gao and DeLuca, 2018). It was therefore concluded that, in these conditions,

P bioavailability was controlled predominantly by abiotic mechanisms related to biochar addition.

5. Conclusions

This study showed that the addition of biochar to second rotation *C. lanceolata* plantation soil enhanced soil P availability, with the effect varying with feedstock type and pyrolysis temperature. Biochar produced from *C. lanceolata* leaves improved soil P availability more than *C. lanceolata* woodchip biochar. Likely explanations for this effect are: 1) direct contribution of soluble P by the leaf biochar itself and of DOC which could have reduced P immobilization; 2) an initial increase in soil pH, thereby reducing the content of sparingly-soluble Al-P; and 3) increased diversity of soil bacterial communities and abundance of P-solubilizing bacteria, resulting from available P and DOC addition in biochar, which may have indirectly improved the soil P availability. However, biochar addition to forest soil had a limited effect on soil phosphatase enzyme activities. Overall, the results demonstrate that conversion of *C. lanceolata* plantation harvest residues into biochar which is recycled back to the soil between rotations could be a viable method to boost soil nutrient availability, particularly P, during subsequent planting. Leaf biochar appears to be more favorable than woodchip biochar for enhancing soil available P in *C. lanceolata* plantation systems. To optimize the use of harvest residues as feedstock for preparation of biochar, different mixtures of leaf and woodchip biochar need to be investigated. As this study was a short-term experiment without plants, long-term field studies of its effect on growth of *C. lanceolata* and life

609 cycle analysis of this biochar use method should be conducted before recommending
610 plantation management with biochar.
611
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617
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Supplementary Material for:

Biochar addition to forest plantation soil enhances phosphorus availability and soil bacterial community diversity

Chuifan Zhou[a], Kate Heal[b], Mulualem Tigabu[c], Lidan Xia[a], Huaying Hu[a], Danyang Yin[a], Xiangqing Ma[a]*

[a]Forestry College, Fujian Agriculture and Forestry University, University Engineering Research Center of Sustainable Plantation Management, Fuzhou, 350002 Fujian Province, People's Republic of China

[b]School of GeoSciences, The University of Edinburgh, Crew Building, Alexander Crum Brown Road, Edinburgh EH9 3FF, UK

[c]Swedish University of Agricultural Sciences, Faculty of Forest Science, Southern Swedish Forest Research Centre, PO Box 49, SE-230 53 Alnarp, Sweden

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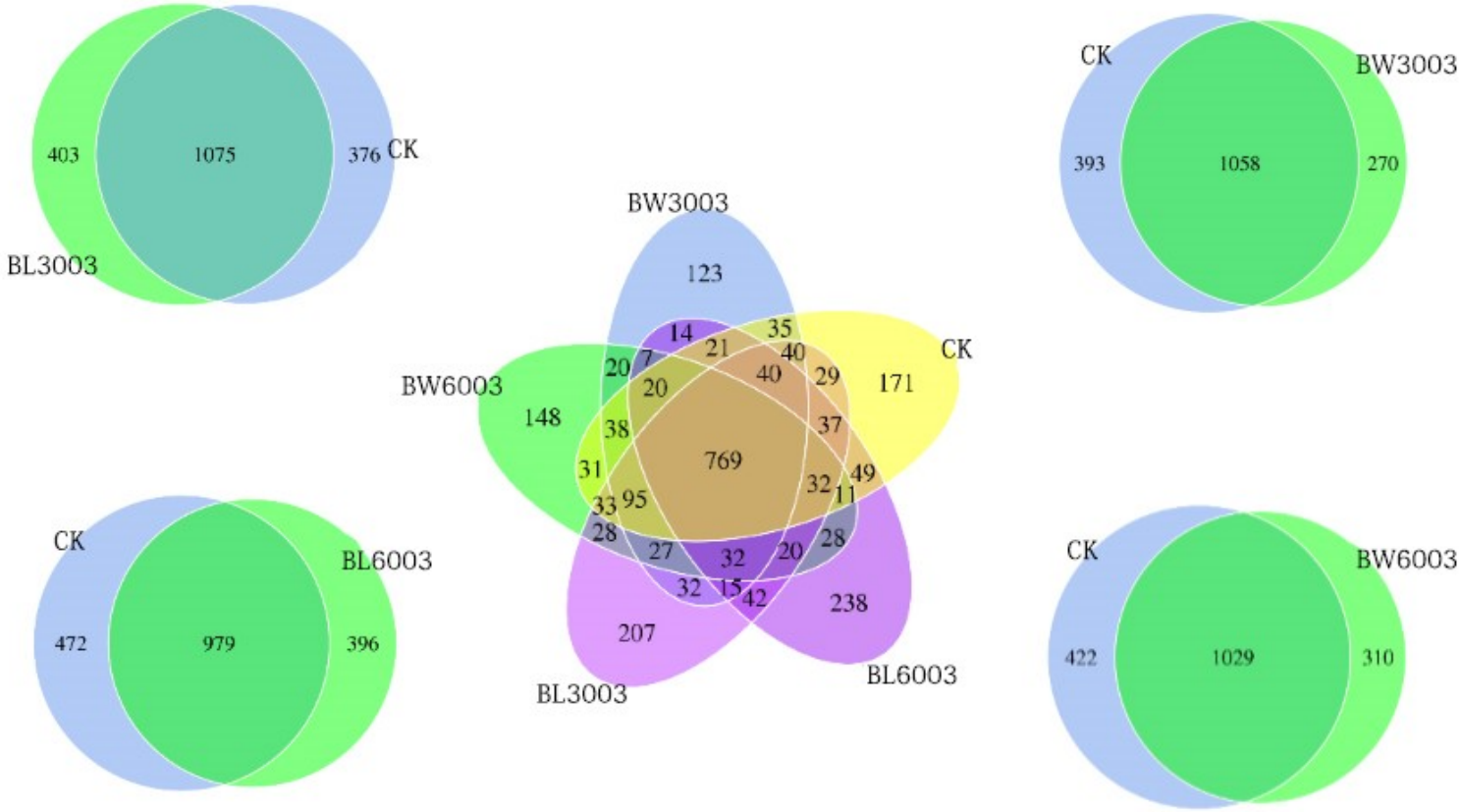

Fig. S1. Venn diagrams of shared OTUs between the biochar-amended soil (3% *w/w*) and the control after 80 days incubation. The treatment abbreviations are shown in Table 1.

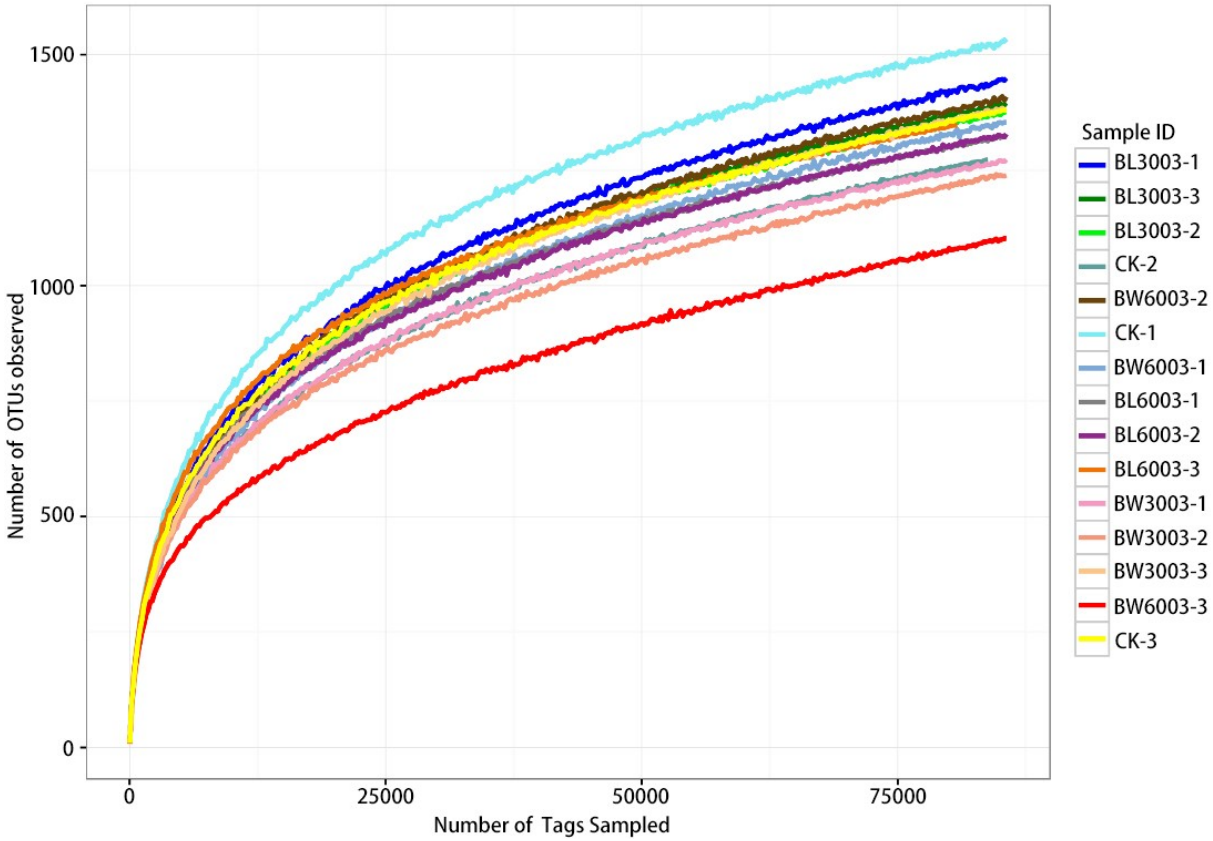

Fig. S2. Rarefaction curve of OTUs for the biochar-amended soil (3% *w/w*) and the control after 80 days incubation. The treatment abbreviations are shown in Table 1. Each of the three replicates for the control and biochar treatments are shown as -1, -2 and -3.

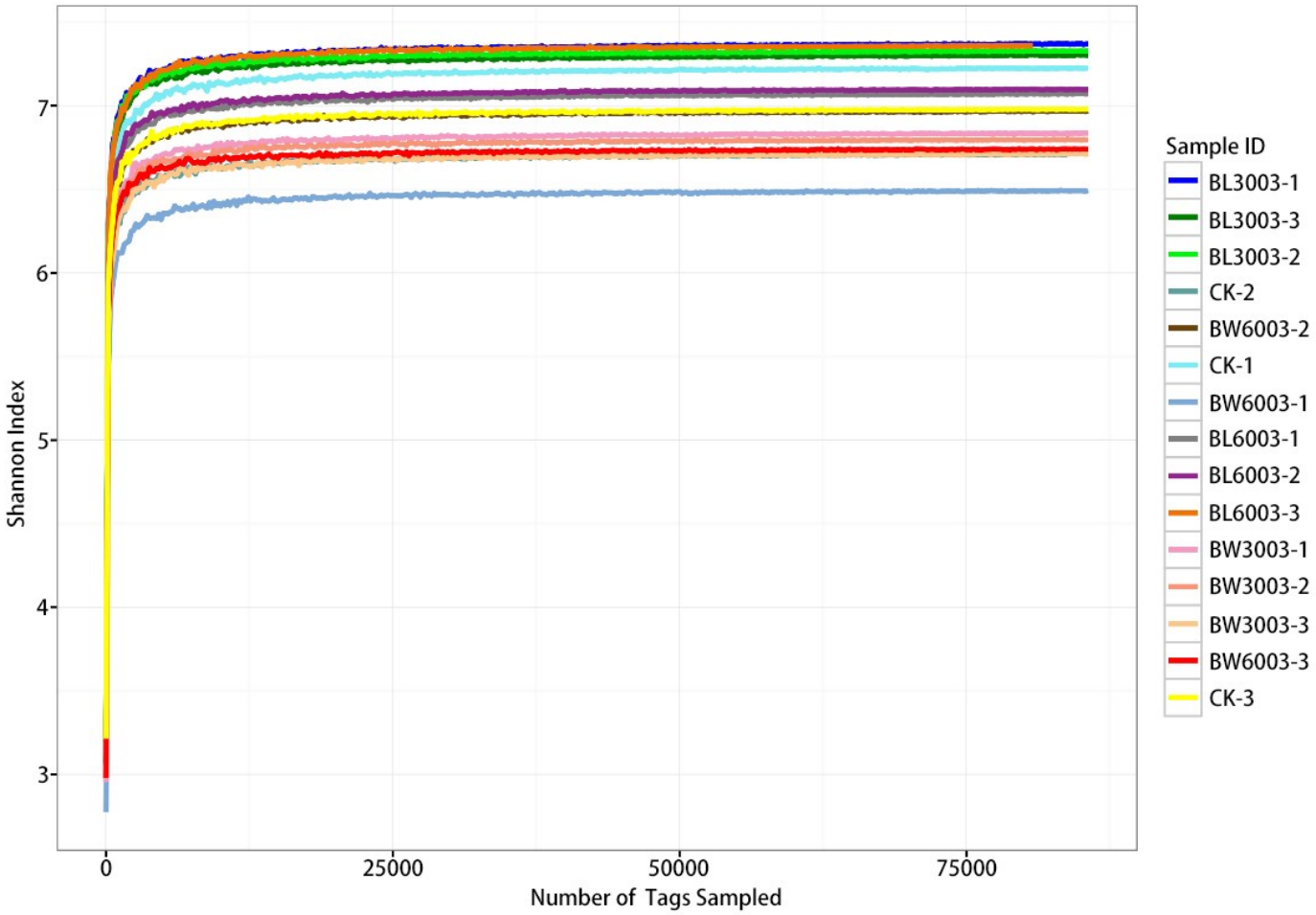

Fig. S3. The Shannon dilution curve of the sample at 0.03 distance. The treatment abbreviations are shown in Table 1. Each of the three replicates for the control and biochar treatments are shown as -1, -2 and -3.

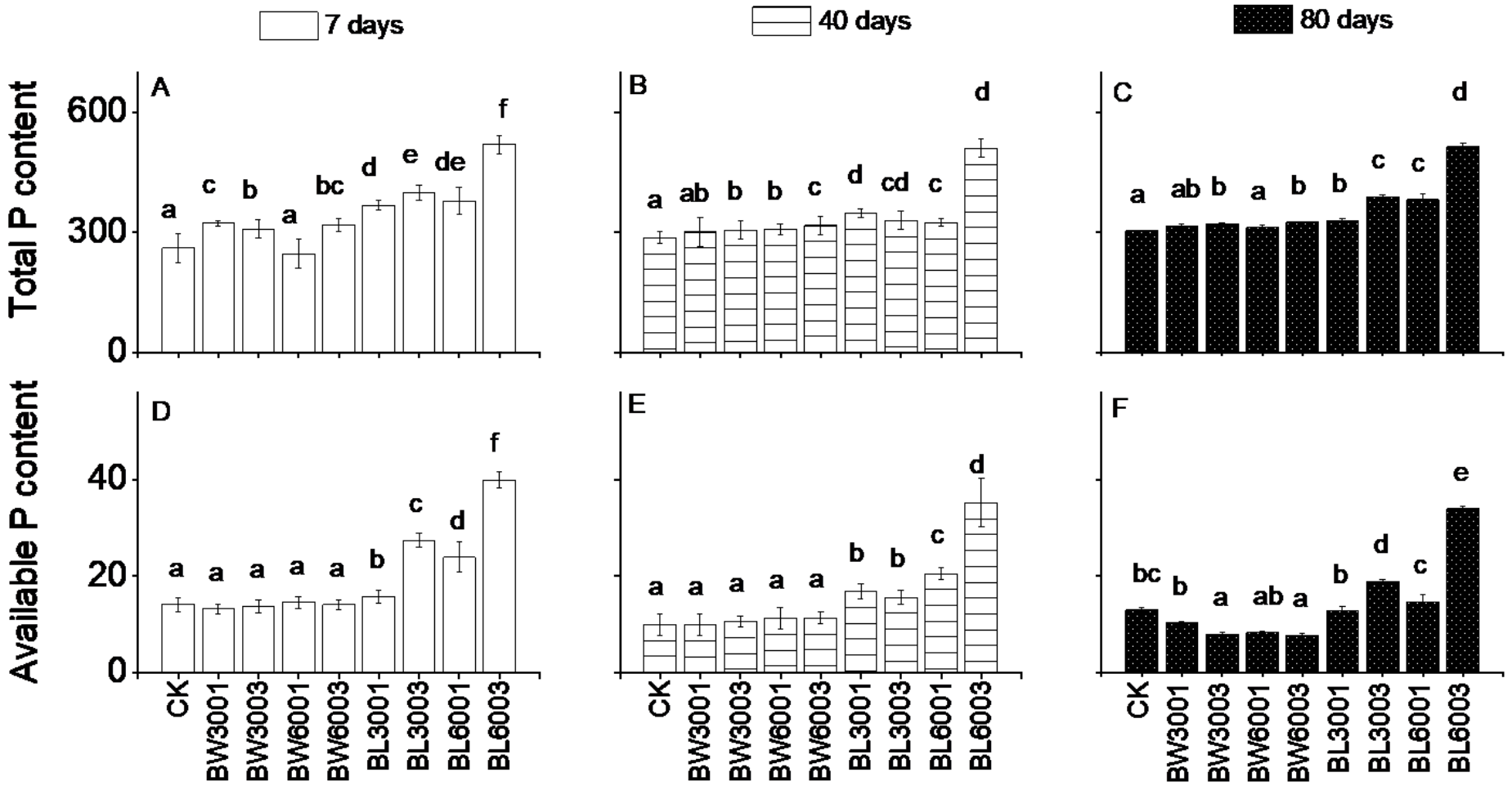

Fig. S4. Soil total phosphorus (A-C) and available phosphorus (D-F) contents (mg kg^{-1}, mean ± SE, n=4) after 7, 40 and 80 days incubation following addition of biochar produced from *C. lanceolata* leaves and woodchips to forest soil. The treatment abbreviations are shown in Table 1. Bars with different letter(s) are significantly different among 80 day incubations ($p < 0.05$).

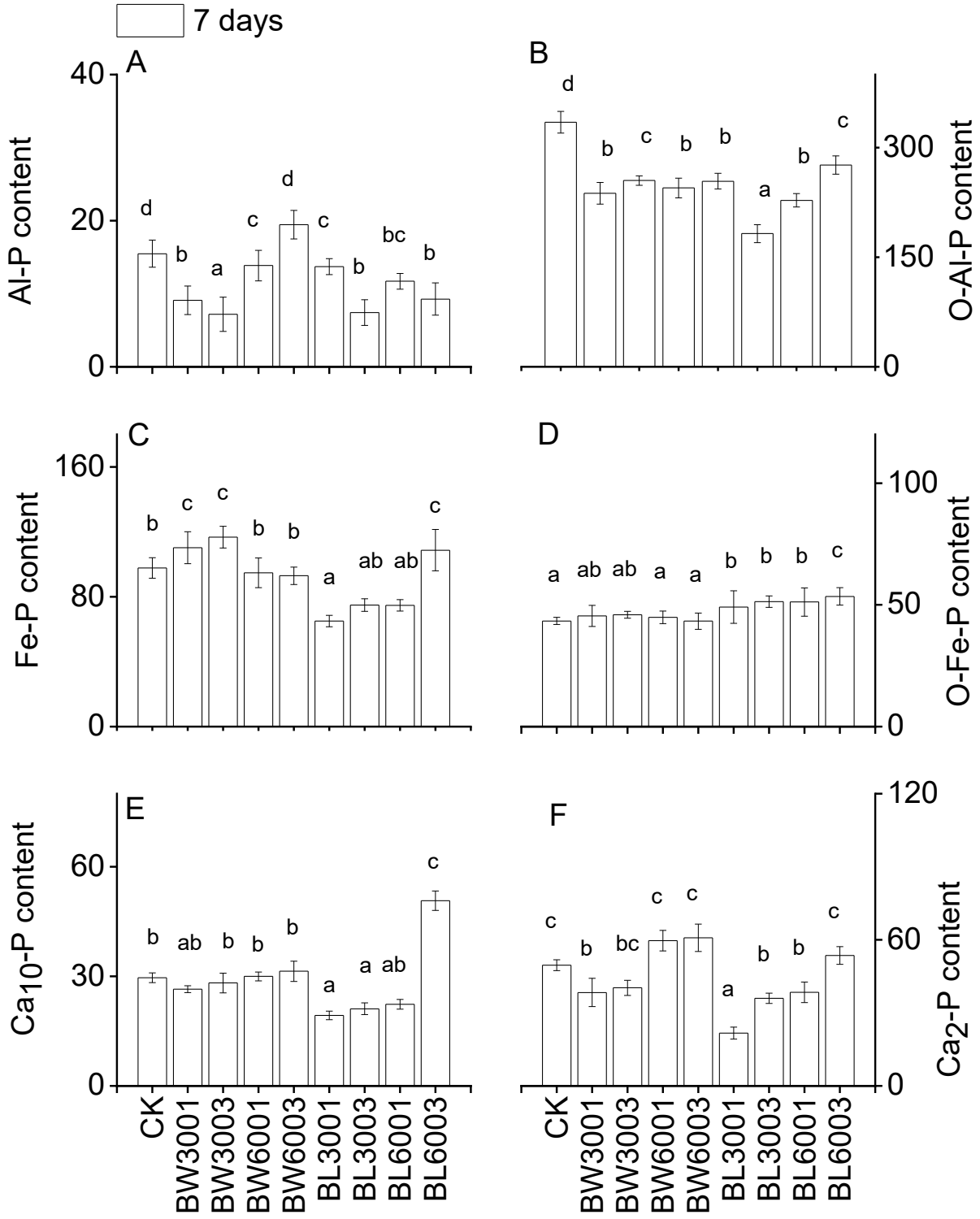

Fig. S5. Soil P fractions (Al-P, Fe-P, O-Al-P, O-Fe-P Ca_2-P, and Ca_{10}-P) contents (mg kg^{-1}, mean ± SE, n=4) after 7 days incubation following addition of biochar produced from *C. lanceolata* leaves and woodchips to forest soil. The treatment abbreviations are shown in Table 1. Bars with different letter(s) are significantly different among treatments ($p < 0.05$). Note different y-axis scales.

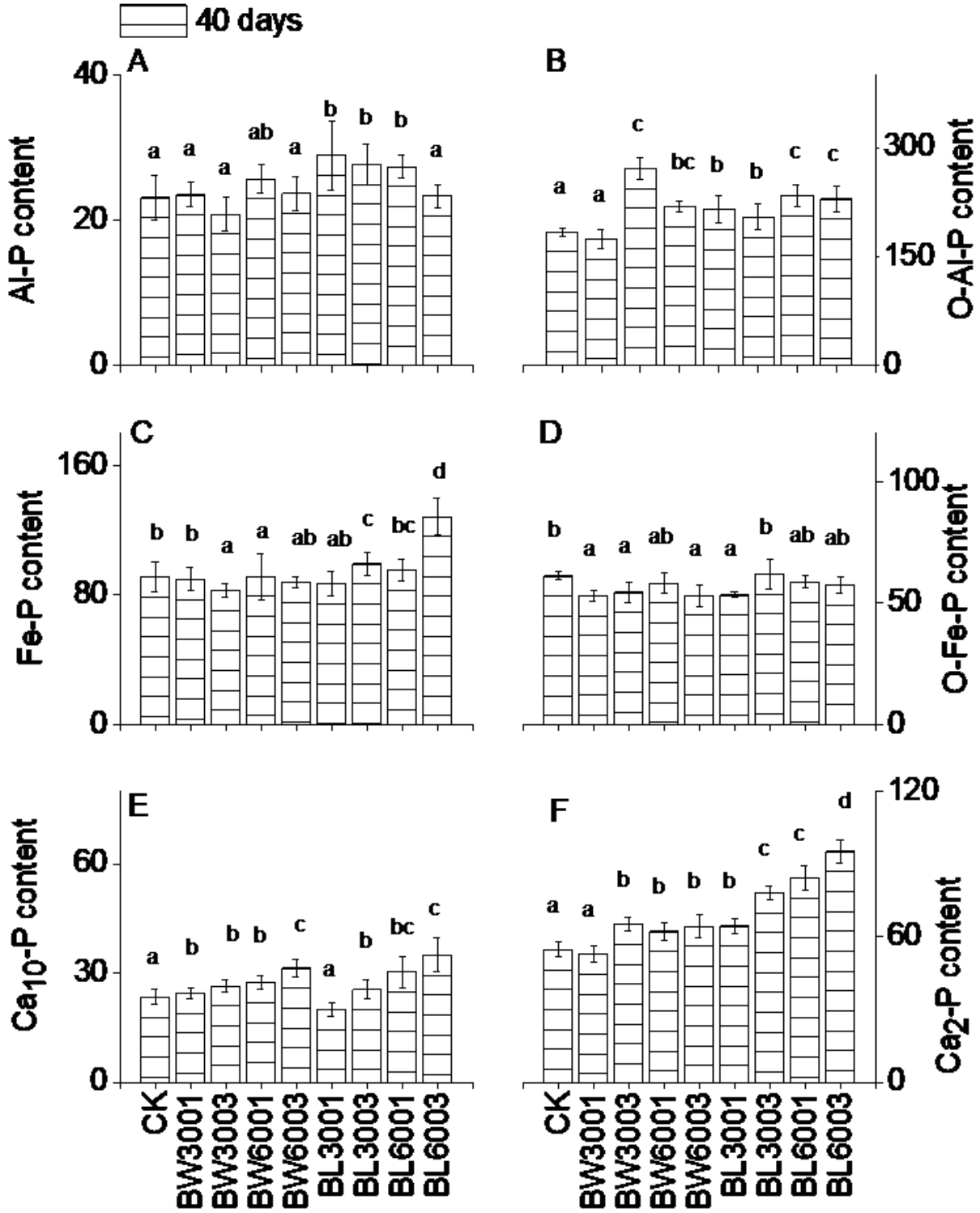

Fig. S6. Soil P fractions (Al-P, O-Al-P, Fe-P, O-Fe-P Ca_2-P, and Ca_{10}-P) contents (mg kg^{-1}, mean ± SE, n=4) after 40 days incubation following addition of biochar produced from *C. lanceolata* leaves and woodchips to forest soil. The treatment abbreviations are shown in Table 1. Bars with different letter(s) are significantly different among treatments ($p < 0.05$). Note different y-axis scales.

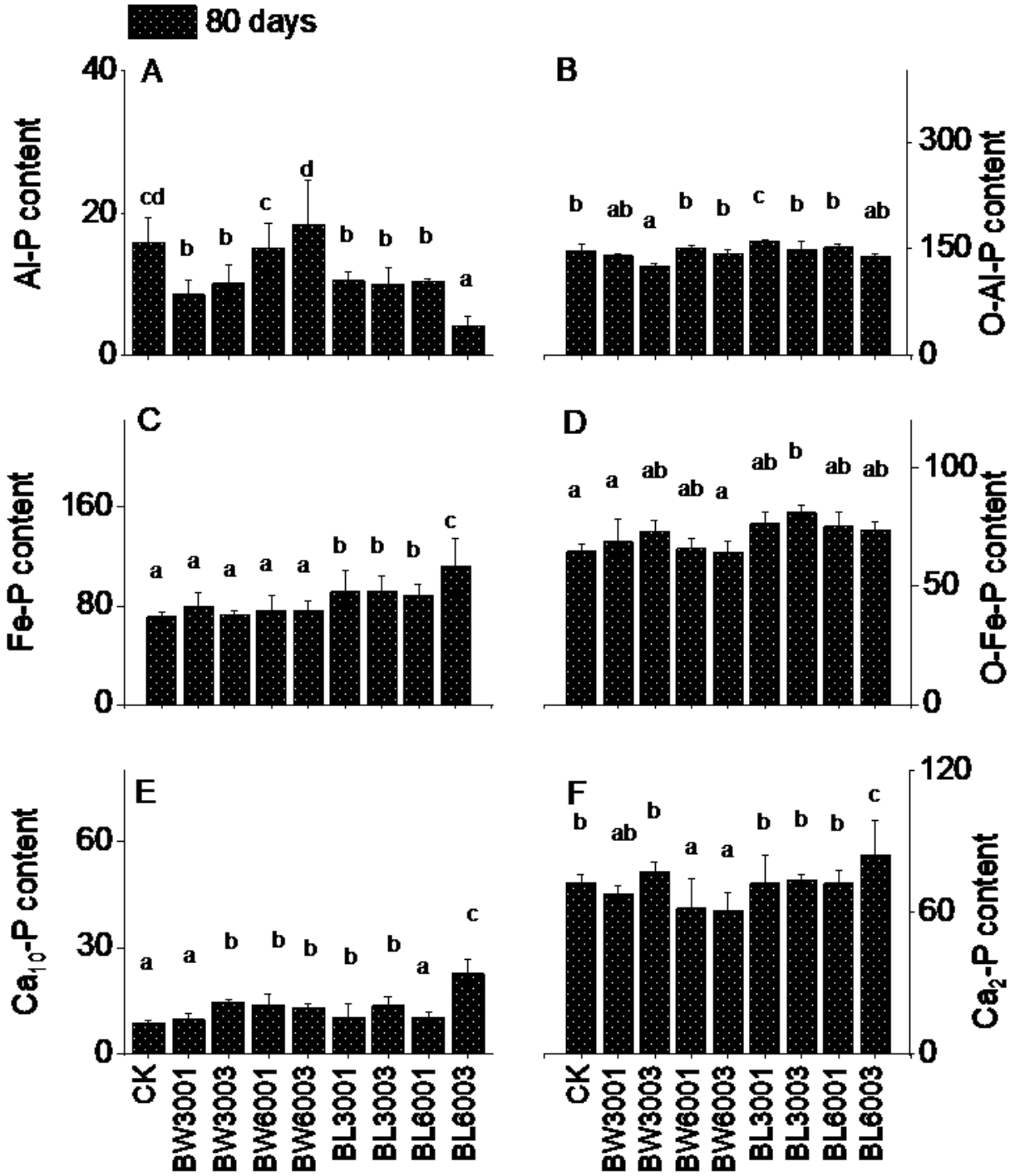

Fig. S7. Soil P fractions (Al-P, O-Al-P, Fe-P, O-Fe-P Ca_2-P, and Ca_{10}-P) contents (mg kg^{-1}, mean ± SE, n=4) after 80 days incubation following addition of biochar produced from *C. lanceolata* leaves and woodchips to forest soil. The treatment abbreviations are shown in Table 1. Bars with different letter(s) are significantly different among treatments ($p < 0.05$). Note different y-axis scales.

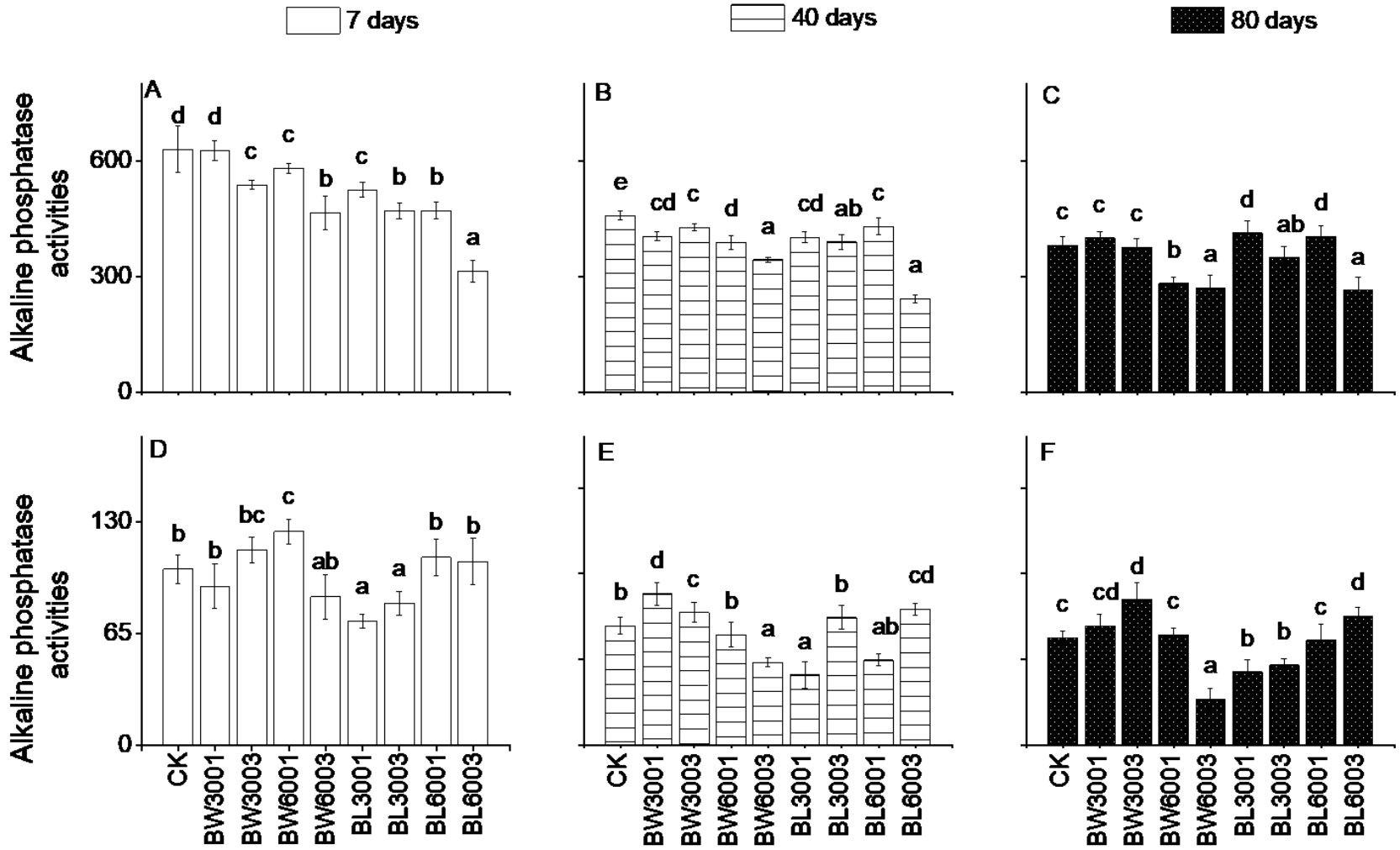

Fig. S8. Soil acid phosphatase (panel A-C) and alkaline phosphatase (panel D-F) activities (mg kg^{-1} h^{-1}, mean ± SE, n=4) after 7, 40 and 80 days incubation following addition of biochar produced from *C. lanceolata* leaves and woodchips to forest soil. The treatment abbreviations are shown in Table 1. Bars with different letter(s) are significantly different among treatments ($p < 0.05$) within each timestep. Note different y-axis scales.

Table S1. Soil bacteria community genus relative abundances (%, mean ± SE, n=3) in the top 25 OTUs for the biochar-amended soil (3% *w/w*) and the control after 80 days incubation following addition of biochar produced from *C. lanceolata* leaves and woodchips to forest soil. Different letter(s) within the same row indicate significant difference between treatments ($p < 0.05$). The treatment abbreviations are shown in Table 1.

Taxonomy	CK	BW3003	BW6003	BL3003	BL6003
Acidothermus	24.96 ± 2.98b	22.9 ± 0.69b	24.71 ± 2.94b	11.6 ± 0.7a	5.68 ± 0.42a
Ktedonobacter	10.05 ± 1.05ab	8.07 ± 0.66ab	11.94 ± 2.24b	5.71 ± 0.28a	6.76 ± 0.33a
Sinomonas	1.17 ± 0.28a	6.45 ± 0.42b	1.76 ± 0.51a	1.31 ± 0.11a	0.89 ± 0.12a
Bradyrhizobium	1.46 ± 0.09a	2.47 ± 0.22b	1.58 ± 0.12a	2.85 ± 0.08b	2.64 ± 0.14b
Exiguobacterium	2.58 ± 0.15a	1.88 ± 0.09a	3.26 ± 0.37a	2.8 ± 0.17a	3.05 ± 0.62a
Burkholderia-Paraburkholderia	0.65 ± 0.03a	1.2 ± 0.11a	1.27 ± 0.4a	6.88 ± 0.58b	1.43 ± 0.21a
Conexibacter	0.28 ± 0.04ab	1.53 ± 0.14c	0.34 ± 0.06ab	0.5 ± 0.08b	0.04 ± 0.009a
Acinetobacter	1.03 ± 0.11a	0.78 ± 0.04a	1.33 ± 0.06a	0.98 ± 0.11a	1.36 ± 0.29a
Planctomyces	1.31 ± 0.33a	1.01 ± 0.1a	1.13 ± 0.43a	2.3 ± 0.45a	6.38 ± 1.28b
Pseudomonas	1.13 ± 0.04a	0.75 ± 0.02a	1.21 ± 0.21a	1.07 ± 0.03a	1.25 ± 0.25a
Sphingomonas	0.98 ± 0.11a	0.9 ± 0.23a	0.51 ± 0.11a	1.26 ± 0.04a	4.87 ± 0.94b
Acidobacterium	0.67 ± 0.07b	0.38 ± 0.08a	0.28 ± 0.03a	0.41 ± 0.02a	0.18 ± 0.04a
Singulisphaera	0.55 ± 0.11a	0.55 ± 0.05a	0.31 ± 0.08a	0.8 ± 0.05a	1.16 ± 0.2b
Candidatus_Solibacter	0.76 ± 0.01b	0.58 ± 0.11ab	0.57 ± 0.05ab	0.41 ± 0.02a	0.44 ± 0.03a
Terracidiphilus	0.34 ± 0.05ab	0.3 ± 0.03ab	0.41 ± 0.08b	0.34 ± 0.01ab	0.15 ± 0.01a
Jatrophihabitans	0.15 ± 0.01a	0.39 ± 0.03b	0.73 ± 0.09b	0.49 ± 0.03b	0.61 ± 0.13b
Sorangium	0.24 ± 0.01b	0.32 ± 0.07b	0.38 ± 0.06b	0.31 ± 0.05b	0.05 ± 0.01a
Candidatus_Xiphinematobacter	1.19 ± 0.21b	0.5 ± 0.12a	0.38 ± 0.13a	0.41 ± 0.07a	0.4 ± 0.06a
Bryobacter	0.33 ± 0.02a	0.28 ± 0.04a	0.19 ± 0.01a	0.22 ± 0.03a	0.35 ± 0.04a
Gemmatimonas	0.18 ± 0.05a	0.15 ± 0.05a	0.33 ± 0.11a	0.26 ± 0.05a	0.79 ± 0.16b
Nocardia	0.05 ± 0.01a	0.08 ± 0.01a	0.08 ± 0.02a	1.15 ± 0.03b	0.05 ± 0.01a
Amycolatopsis	0.08 ± 0.02a	0.06 ± 0.01a	0.05 ± 0.01a	1.12 ± 0.49b	0.06 ± 0.01a
Paucimonas	0.02 ± 0.001a	0.02 ± 0.002a	0.02 ± 0.005a	1.91 ± 0.14b	0.03 ± 0.004a
Bacteroides	1.29 ± 1.25a	0.007 ± 0.002a	0.01 ± 0.008a	0.03 ± 0.02a	0.65 ± 0.6a
Niastella	0.01 ± 0.004a	0.004 ± 0.001a	0.004 ± 0.001a	0.005 ± 0.001a	1.73 ± 0.46b
Total abundance	51.47	51.56	52.78	45.13	41.00

Table S2. Soil pH (mean ± SE, n=4) for the control and biochar-amended soil at different times during the soil incubation experiment. Different letter(s) within the same row indicate significant difference between treatments ($p < 0.05$). The treatment abbreviations are shown in Table 1.

Days from start of experiment	CK	BW3001	BW3003	BW6001	BW6003	BL3001	BL3003	BL6001	BL6003
7	4.41 ± 0.03de	4.38 ± 0.03e	4.33 ± 0.03e	4.54 ± 0.02d	4.73 ± 0.04c	4.73 ± 0.07c	4.97 ± 0.08b	5.07 ± 0.02b	6.14 ± 0.10a
40	4.36 ± 0.01d	4.39 ± 0.05d	4.32 ± 0.04d	4.44 ± 0.03d	4.67 ± 0.07cd	4.96 ± 0.21bc	4.85 ± 0.26bc	5.14 ± 0.17b	5.95 ± 0.07a
80	4.23 ± 0.02cd	3.97 ± 0.01d	4.05 ± 0.04d	4.03 ± 0.05d	4.81 ± 0.55b	4.17 ± 0.03cd	4.63 ± 0.06bc	4.42 ± 0.01bcd	5.54 ± 0.04a